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ANNUAL REPORT
FY 1979

NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES

NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND

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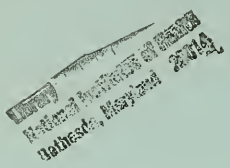

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THE DIRECTOR'S REPORT

Fiscal year 1979 marks the fifth year that I have had the privilege of serving as Director of the National Institute of General Medical Sciences. During this year, the Institute consolidated the changes made as a result of the reorganization in 1978 and reaffirmed, through the funding of a new special program, its commitment to the support of the basic research which undergirds the program of the categorical Institutes.

Pharmacology-Toxicology Program - For ten years, this program was ably directed by Dr. Byron B. Clark. In January, 1979, Dr. Clark retired and it is a pleasure to announce that Dr. Sara Gardner was appointed as Director of the program.

Visiting Scientific Staff - Program staff are in close communication with the investigators in the field and, at irregular intervals, such individuals visit the Institute, present seminars and generally advise the staff regarding important scientific developments. In the field of pharmacology, the Institute has been exceedingly fortunate to have Dr. Folke Sjöqvist, Professor and Chairman of Clinical Pharmacology, Karolinska Institute, Stockholm, Sweden, serve as a consultant and Special Assistant to the Director for the past year. His expertise and advice have been particularly important in the revitalization of the pharmacology program, at this crucial point in its history, and in the establishment of a clinical pharmacology research training program as part of the only NIGMS intramural activity, namely, the Pharmacology Research Associate Training (PRAT) Program.

Shared Instrumentation Program - The staff of the Institute has been cognizant, for some time, of the lack in some cases, or the obsolescence, in others, of sophisticated analytical instrumentation at many of the universities and institutions to which research grants have been awarded both by the many components of the National Institutes of Health and by, for example, the National Science Foundation. In addition, since many investigators do not utilize such instruments on a full-time basis, it was felt that the idea of sharing such very expensive instruments should be fostered. Therefore, as described in the report of the Physiology and Biomedical Engineering Program, in the spring of 1979, the Institute issued a Request for Grant Applications for a Shared Instrumentation Program for NIGMS grantees (Attachment A).

Two hundred and twelve applications were received from grantees of all the Institute's programs. With the excellent cooperation of the Division of Research Grants and the thoughtful interactions on the part of members of the staffs of all the Institute's programs, the review of these applications and award of 65 grants was accomplished efficiently and within a very short time-frame. Awards were distributed widely among the various instruments, as well as geographically, and took into consideration the needs of the institutions and the investigators. Attachments B and C list the awards.

Program Initiatives - The Institute feels that a vital factor in optimizing available grant resources is the competence of its staff of health scientist administrators who have close contact with investigators supported by NIGMS. To remain competent, these administrators must stay abreast of the most recent advances in their respective areas of scientific expertise through reading, attendance at scientific meetings, visits to grantee institutions and close association with the intramural NIH scientists. The staff of each program also is encouraged to hold workshops and develop new initiatives in order to assess and fulfill the needs of the scientific community. These activities were very successful during this past year and are described individually in the individual program reports.

Office of Review Activities - The duties of this office have continued to evolve as it undertook an enormous workload related to the receipt of the very large number of renewal applications for the National Research Service institutional fellowship awards, first funded in 1975.

Office of Recombinant DNA Activities (ORDA) - The NIGMS supports more than 80% of all the research supported by NIH which involves the use of recombinant DNA technology. The Director, NIGMS, thus, had been concerned for some time, regarding the appropriateness of having the responsibility for both the support and the "regulation" of such research. Furthermore, other activities related to use of recombinant DNA technology, such as risk assessment and the development of P4 facilities, were assigned to the National Institute of Allergy and Infectious Diseases (NIAID). Therefore, at my recommendation, the Director, NIH, transferred ORDA to NIAID at the end of FY 1979. Close interaction with NIGMS staff must continue, of course, because of the nature of the grants supported by the Institute.

Staff Changes

New Professional members of the staff:

Dr. Christine Carrico - Pharmacology-Toxicology Program
Dr. Robert Gulley - Genetics Program

Staff Appointments

Dr. W. Sue Badman - Chief, Instrumentation Section, Physiology and Biomedical Engineering Program

Dr. Marvin Cassman - Chief, Molecular Basis of Disease Section, Cellular and Molecular Basis of Disease Program

Dr. Sara Gardner - Director, Pharmacology-Toxicology Program

Dr. Vincent Price - Acting Director, Office of Review Activities

Staff Departures

Mrs. Emma Lou Akers
Dr. John Dalton
Dr. Winfred Harris
Dr. Prince Rivers
Dr. Anthony Zavadil

National Advisory General Medical Sciences Council

Drs. Francisco Ayala and Sarah Ratner and Ms. Joanne Mizner were appointed for four-year terms.

REQUEST FOR RESEARCH GRANT APPLICATION: RFA

TITLE: NIGMS SHARED INSTRUMENTATION PROGRAM | ANNOUNCEMENT

The National Institute of General Medical Sciences announces to its current grantees a one-time competitive program to provide funds for the purchase of new, or the updating of existing, major analytical research instruments which might not be justified fully for a single project, but which could serve several projects on a shared basis. The primary intent is to provide NIGMS grantees with better access to modern instrumentation. A secondary goal is to ensure that new instrumental techniques become widely disseminated among potential users. Access to the equipment provided by this program therefore will not be limited to NIGMS grantees, although the needs of NIGMS grantees will be the primary basis for determining which awards are to be made.

Applications will be accepted for a single deadline of March 15, 1979. No additional competitions are planned.

Support Provided by the Grant

Requests should be for a single analytical instrument or instrument system, or for funds to upgrade an existing instrument. Examples of the kind of instruments which can be provided or upgraded by this program are electron microscopes, fluorescent cell sorters, nuclear magnetic resonance spectrometers, and combined gas chromatograph-mass spectrometers. Excluded from consideration under this program are routine preparative instruments. Requests for general purpose computer facilities also will not be considered. The cost of the equipment requested is expected to fall in the range of $40,000-$150,000. Maintenance costs and support for technical assistance may be provided to a maximum of $15,000 per year for up to three years only. It is expected that at least one half of the costs for technical support personnel be assumed by the applicant institution. No funds for installation or renovation will be provided. The first-year award will be for expenses related directly to the purchase or upgrading of equipment as well as the maintenance support mentioned above. Future year commitments will be for support funds only.

Eligibility

The principal investigator on the application must hold an active NIGMS research grant. This individual must have clearly demonstrated expertise in the use of the type of instrument requested, and must assume responsibility for the equipment and for all related administrative functions. Other named investigators on the application will be members of the core user group (see below). All such individuals must have currently funded NIH research grants. At least one half of the core user group must hold NIGMS research grants.

Preparation

Use form PHS 398, available at your institution, for applying. Across the top of the application face page write: NIGMS SHARED INSTRUMENTATION PROGRAM. Both a core user group and a potential user group should be identified. The core user group will consist of the principal investigator plus two to four other named investigators. The proposal should identify the specific research projects of these investigators which require the instrument being requested, together with an estimate of the percentage use each will require. It is expected that the core user group will account for at most 75 percent of the instrument use time. The remaining instrument time should be made available to other qualified scientists in the geographical vicinity. The application should document the existence of such a potential user group which would benefit from occasional access to the instrument. The latter investigators need not be NIH grantees.

The justification should state whether similar instruments are available on or near the campus and, if so, why an additional instrument is required. No more than one instrument of any particular kind will be awarded to any single institution or campus.

Up to 50 awards will be made provided enough applications of high quality are received and contingent on the availability of funds. Assurance is required that the equipment will be operated as a shared facility. In addition to progress reports detailing the contribution of the instrument to the research of the core user group, it is expected that a list of outside users and the instrument time made available to them will be submitted annually for a period of three years.

Criteria for Award

The following factors will be considered in the review:

1) Justification of need for the instrument;

2) Scientific merit of the research for which the instrument is required;

3) Expertise of the principal investigator in the use of the instrument;

4) Institutional commitment to help in providing support staff and maintenance;

5) Adequate administrative arrangements for use of the instrument.

Other factors, beyond the scientific review, will affect the award process. NIGMS serves a broad community of investigators in many diverse areas of biomedical research. The awards will not be restricted to some limited area of instrumentation, so that this program can serve a wide spectrum of NIGMS grantees. In addition, given the goals of this program, some geographical

factors will be considered in making the final awards. For example, NIGMS will avoid placing two or more similar instruments in an area serving the same potential user population. Similarly, the absence of existing facilities will clearly be a factor. With these caveats, the scientific merit of the research projects for which instrumentation is needed will, as always, be a major consideration.

Prior to submission of applications, contact should be made with:

Dr. Marvin Cassman
National Institute of General
Medical Sciences, NIH

Phone: (301) 496-7463

Receipt and Review

Applications should be sent to: Division of Research Grants
National Institutes of Health
Room 240, Westwood Building
5333 Westbard Avenue
Bethesda, Maryland 20014

and must be received no later than March 15, 1979, in order to be considered for review.

Review will be arranged by the Division of Research Grants, NIH. Awards will be made in September 1979.

SHARED INSTRUMENTATION AWARDS
BY STATE AND INSTITUTION

CALIFORNIA	
California Institute of Technology (Dickerson)	Computer Graphics
Scripps Clinic and Research Foundation (Whiteley)	Amino Acid Analyzer
Stanford University (Herzenberg)	Cell Sorter
University of California, Berkeley (Bartlett)	90 MHz FT-NMR
University of California, Los Angeles (Lake)	Electron Microscope
University of California, San Diego (Nyhan)	GC/MS
University of California, San Francisco (James)	200 MHz NMR
University of California, San Francisco (Hunt)	Laser Light Scattering
University of Southern California (Stephens)	CD
University of Southern California (Olah)	200 MHz NMR
COLORADO	
University of Denver (Wickstrom)	CD
CONNECTICUT	
University of Connecticut Health Center (Fernandez)	Fluorescence Microscope
Yale University (Palade)	Electron Microscope
DISTRICT OF COLUMBIA	
Carnegie Institution of Washington D.C. (Ward)	Electron Microscope

FLORIDA	
Papanicolaou Cancer Research Institute (Meloche)	90 MHz FT-NMR
University of South Florida (Solomonson)	EPR
ILLINOIS	
Southern Illinois University, Carbondale (Borgia)	Protein Sequencer
University of Chicago (Law)	Amino Acid Analyzer
University of Chicago (Strauss)	Electron Microscope
University of Illinois, Urbana-Champaign (Dunn)	Ultrasonics
INDIANA	
Purdue University (Rossman)	X-Ray Film Scanner
IOWA	
Iowa State University (Kraus)	200 MHz NMR
University of Iowa (Blakley)	Protein Sequencer/Amino Acid Analyzer
KANSAS	
University of Kansas (Hanzlik)	GC/MS
MAINE	
Jackson Laboratory, Bar Harbor (Chen)	Cell Sorter
MARYLAND	
Johns Hopkins University (Amzel)	X-Ray Film Scanner
Johns Hopkins University (Ts´o)	200 MHz NMR

MASSACHUSETTS	
Brandeis University (Rosenblum)	90 MHz NMR
Massachusetts General Hospital (Burke)	GC/MS
Massachusetts Institute of Technology (Whitesides)	FT-IR
Tufts University (Goldberg)	Electron Microscope
MICHIGAN	
University of Michigan (Williams)	HPLC and Sequencer Accessories
MINNESOTA	
University of Minnesota at Minneapolis (Hogenkamp)	90 MHz FT-NMR
MISSOURI	
Washington University (Goodenough)	Scanning Electron Microscope
NEW YORK	
Albany Medical College (Kaplan)	Gamma Scintillation Camera
Columbia University (Breslow)	GC/MS
Columbia University (Cantor)	Cell Sorter
Cornell University (Webb)	Fluorescent Microscope
Cornell University Medical Center (Breslow)	CD
Rockefeller University (Luck)	Electron Microscope
State University of New York at Albany (Zubieta)	X-Ray Diffractometer

NEW YORK (CONTINUED)

University of Rochester (Olmsted)	Electron Microscope
Yeshiva University, Albert Einstein Medical School (Satir)	Electron Microscope
Yeshiva University, Albert Einstein Medical School (Steinman)	Protein Sequencer

NORTH CAROLINA

Duke University (Nicklas)	Electron Microscope

OHIO

Case Western Reserve University (Urbach)	EPR
Case Western Reserve University (Stambrook)	Electron Microscope
Ohio State University (Horton)	200 MHz NMR

OREGON

University of Oregon (Hudson)	Laser
University of Oregon (Schellman)	CD

PENNSYLVANIA

Carnegie-Mellon University (Llinas)	200 MHz NMR
Pennsylvania State University (Villafranca)	200 MHz NMR
University of Pennsylvania (Cohn)	Console for 220 MHz NMR
University of Pennsylvania (Chance)	EXAFS Optics

TENNESSEE	
University of Tennessee, Knoxville (Becker)	Fluorescence Microscope
Vanderbilt University (Williams)	Electron Microscope
Vanderbilt University (Watson)	GC/MS
TEXAS	
Baylor College of Medicine (Bryan)	Cell Sorter
University of Texas Hlth. Sci. Ctr., Dallas (Estabrook)	GC/MS
University of Texas Hlth. Sci. Ctr., San Ant. (Horowitz)	CD
VIRGINIA	
University of Virginia (Thompson)	Fluorometer
WASHINGTON	
University of Washington (Walsh)	Protein Sequencer/Amino Acid Analyzer
Washington State University (McFadden)	Amino Acid Analyzer
WEST VIRGINIA	
West Virginia University (Wimmer)	GC/MS
WISCONSIN	
University of Wisconsin, Madison (Kahan)	Cell Sorter

SHARED INSTRUMENTATION AWARDS
BY INSTRUMENT

NMR

1.	Bartlett	UC, Berkeley, California	141,483
2.	Cohn	University of Pennsylvania	143,530
3.	Hogenkamp	University of Minnesota	143,711
4.	Horton	Ohio State University	165,000
5.	James	UC, San Francisco, California	164,850
6.	Kraus	Iowa State University	165,000
7.	Llinas	Carnegie Mellon, Pennsylvania	165,000
8.	Meloche	Papanicolaou Institute, Florida	145,840
9.	Olah	University of Southern California	165,000
10.	Rosenblum	Brandeis, Massachusetts	52,240
11.	Ts´o	Johns Hopkins University, Maryland	162,925
12.	Villafranca	Pennsylvania State University	155,929

ELECTRON MICROSCOPES

1.	Goldberg	Tufts University, Massachusetts	102,520
2.	Goodenough	Washington University, Missouri	66,470
3.	Lake	UC, Los Angeles, California	109,540
4.	Luck	Rockefeller University, New York	102,413
5.	Nicklas	Duke University, North Carolina	103,440
6.	Olmsted	University of Rochester, New York	117,121
7.	Palade	Yale University, Connecticut	165,000
8.	Satir	Albert Einstein Med. School, New York	165,000
9.	Stambrook	Case Western Reserve, Ohio	97,947
10.	Strauss	University of Chicago, Illinois	103,961
11.	Ward	Carnegie Institution of Washington, D.C.	85,500
12.	Williams	Vanderbilt University, Tennessee	87,000

SPECTROSCOPY

1.	Breslow	Cornell Medical School, New York	61,675
2.	Horowitz	U. of Texas Hlth. Sci. Center, Houston	53,700
3.	Hudson	University of Oregon	113,294
4.	Hunt	UC, San Francisco, California	67,801
5.	Schellman	University of Oregon	50,285
6.	Stephens	University of Southern California	64,501
7.	Thompson	University of Virginia	54,700
8.	Webb	Cornell University, New York	91,000
9.	Whitesides	Massachusetts Institute of Technology	126,098
10.	Wickstrom	University of Denver, Colorado	50,350

PROTEIN SEQUENCERS AND AMINO ACID ANALYZERS

1.	Blakley	University of Iowa	146,005
2.	Borgia	Southern Illinois University	73,144
3.	Law	University of Chicago, Illinois	104,977

PROTEIN SEQUENCERS AND AMINO ACID ANALYZERS (CONTINUED)

4.	McFadden	Washington State University	98,800
5.	Steinman	Albert Einstein, New York	117,198
6.	Walsh	University of Washington	148,298
7.	Whiteley	Scripps Clinic & Resch. Fdn., California	69,062
8.	Williams	University of Michigan	61,600

MASS SPECTROSCOPY

1.	Breslow	Columbia University, New York	165,000
2.	Burke	Massachusetts General Hospital	146,784
3.	Estabrook	U. of Texas Hlth. Sci. Center, Dallas	158,625
4.	Hanzlik	University of Kansas	146,800
5.	Nyhan	UC, San Diego, California	163,349
6.	Watson	Vanderbilt University, Tennessee	157,880
7.	Wimmer	West Virginia University	147,000

X-RAY AND COMPUTER GRAPHICS

1.	Amzel	Johns Hopkins University, Maryland	92,412
2.	Chance	University of Pennsylvania	131,557
3.	Dickerson	California Institute of Technology	126,332
4.	Rossman	Purdue University, Indiana	51,393
5.	Zubieta	SUNY - Albany, New York	142,000

CELL SORTERS

1.	Bryan	Baylor University, Texas	125,600
2.	Cantor	Columbia University, New York	147,825
3.	Chen	Jackson Lab., Bar Harbor, Maine	100,740
4.	Herzenberg	Stanford University, California	148,577
5.	Kahan	University of Wisconsin	38,534

LIGHT AND FLUORESCENCE MICROSCOPY

1.	Becker	University of Tennessee	105,108
2.	Fernandez	University of Connecticut Health Center	149,557

EPR

1.	Solomonson	University of South Florida	52,600
2.	Urbach	Case Western Reserve University	89,789

ULTRASONICS

1.	Dunn	University of Illinois	50,155

RADIOLOGY

1.	Kaplan	Albany Medical College, New York	150,400

FY 1979 TOTAL SUPPORT - BY ACTIVITY/GRANT TYPE
AND SHOWING PROGRAM AREAS
(Dollars in thousands)

		CMBD		GEN		P/T		PBME		MARC		TOTAL	
		NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
P01	TYPES 5 & 7	6	2,163	6	2,607	3	967	7	2,665			22	8,402
	TYPES 2 & 9	2	1,121	1	321	1	161	4	1,434			8	3,036
	TYPE 1	1	528			2	1,055					3	1,583
	TYPE 3			1	230	1	60	2	120			4	410
		9	3,812	8	3,158	7	2,243	13	4,219			37	13,431
P41	TYPE 1							65	7,638			65	7,638
R01	TYPES 5 & 7	512	37,047	553	43,210	145	11,938	136	11,554			1,346	103,750
	TYPES 2 & 9	170	13,334	141	12,679	59	3,873	44	4,738			414	34,624
	TYPE 1	192	11,651	180	12,582	104	5,945	76	5,277			552	35,455
	TYPE 3	25	564	27	603	2	43	7	303			61	1,513
		899	62,597	901	69,075	310	21,799	263	21,872			2,373	175,343
R23	TYPES 5 & 7							10	367			10	367
	TYPE 1							10	387			10	387
								20	754			20	754
RESEARCH PROJECTS		908	66,409	909	72,232	317	24,042	361	34,482			2,495	197,165
P50	TYPES 5 & 7	1	640	4	3,544	4	2,883	11	6,763			20	13,832
	TYPES 2 & 9			1	1,009	2	1,179	2	1,152			5	3,341
	TYPE 1					1	273	1	457			2	731
	TYPE 3			1	225	1	47	1	53			3	325
CENTERS		1	640	6	4,778	8	4,383	15	8,426			30	18,228
R13	TYPE 1	3	21			1	56	2	8			6	85
TOTAL RESEARCH		912	67,070	915	.77,011	326	28,482	378	42,916			2,531	215,478

NOTE: Discrepancies in totals due to rounding.

FY 1979 TOTAL SUPPORT BY ACTIVITY/GRANT TYPE
AND SHOWING PROGRAM AREAS
(Dollars in thousands)

	CMBD		GEN		P/T		PBME		MARC		TOTAL	
	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT	NO.	AMOUNT
K04 TYPES 5 & 7	38	1,383	55	1,925	11	419	15	558			119	4,284
TYPE 3	2	8					1	1			3	10
	40	1,391	55	1,925	11	419	16	559			122	4,294
K06 TYPES 5 & 7	8	256	7	226	3	90	4	128			22	701
TOTAL CAREER	48	1,647	62	2,151	14	509	20	687			144	4,994
F32 TYPES 5 & 7	51	662	65	879	10	111	2	34			128	1,685
TYPES 2 & 9	2	24									2	24
TYPE 1	126	1,698	136	1,835	24	321	10	141			296	3,995
TYPE 3	4	8	4	1							8	9
	183	2,392	205	2,715	34	432	12	175			434	5,713
F34 TYPES 5 & 7									13	254	13	254
TYPES 2 & 9									3	34	3	34
TYPE 1									18	371	18	371
TYPE 3									1	5	1	5
									35	664	35	664
TOTAL FELLOWSHIPS	183	2,392	205	2,715	34	432	12	175	35	664	469	6,377
T01 TYPES 5 & 7	1	48									1	48
T32 TYPES 5 & 7	89	19,638	56	6,358	63	5,106	42	4,049	17	1,319	267	36,470
TYPE 1	7	1,308	7	679	4	311	5	407	9	887	32	3,592
TYPE 3	1	69	1	3							2	72
	97	21,016	64	7,040	67	5,417	47	4,456	26	2,206	301	40,135
TOTAL TRAINING	98	21,063	64	7,040	67	5,417	47	4,456	26	2,206	302	40,183
FINAL TOTAL GRANTS	1,241	92,172	1,246	88,916	441	34,840	457	48,234	61	2,870	3,446	267,032

NOTE: Discrepancies in totals due to rounding.

FY 1979 NATIONAL RESEARCH SERVICE AWARDS TOTAL SUPPORT
BY ACADEMIC LEVEL/ACTIVITY/ANNOUNCED AREA
(Dollars in thousands)

ANNOUNCED AREA	TYPES 5 & 7		TYPES 2 & 9		TYPE 1		TYPE 3		TOTAL	
	NO. AWDS.	DOLLARS	NO. AWDS.	DOLLARS	NO. AWDS.	DOLLARS	NO. AWDS.	DOLLARS	NO. AWDS.	DOLLARS
PREDOCTORAL INDIVIDUAL (F34)										
MARC	9	157	1	16	10	197	1	5	21	375
MARC (Paid by other Insts.)	(7	121)			(1	13)			(8	134)
POSTDOCTORAL INDIVIDUAL (F32, F34, F36)										
PATH	1	14							1	14
CELL-MOL	50	648	2	24	126	1,698	4	8	182	2,378
GEN	65	879			136	1,835	4	1	205	2,715
PHARM-SCI	10	111			24	321			34	432
SYS-INT	2	34			4	54			6	88
TRAUMA					3	46			3	46
ANES					1	13			1	13
BEHAV-SCI					2	28			2	28
MARC	4	97	2	18	8	174			14	289
MARC (Paid by other Insts.)	(3	58)			(4	104)	(1	0.5)	8	162
TOTAL	132	1,782	4		304	4,169	8	9	44	6,002

NOTE: Discrepancies in totals due to rounding.

FY 1979 NATIONAL RESEARCH SERVICE AWARDS TOTAL SUPPORT
BY ACADEMIC LEVEL/ACTIVITY/ANNOUNCED AREA
(Dollars in thousands)

ANNOUNCED AREA	TYPES 5 & 7 NO. AWDS.	TYPES 5 & 7 NO. POS.	TYPES 5 & 7 DOLLARS	TYPES 2 & 9 NO. AWDS.	TYPES 2 & 9 NO. POS.	TYPES 2 & 9 DOLLARS	TYPE 1 NO. AWDS.	TYPE 1 NO. POS.	TYPE 1 DOLLARS	TYPE 3 NO. AWDS.	TYPE 3 NO. POS.	TYPE 3 DOLLARS	TOTAL NO. AWDS.	TOTAL NO. POS.	TOTAL DOLLARS
PREDOCTORAL INSTITUTIONAL (T32)															
CELL-MOL	51	1,233	11,106				4	28	290	1	7	69	56	1,268	11,466
GEN	34	499	4,422				6	63	587				40	562	5,009
PHARM-SCI	48	463	4,170				4	37	311				52	500	4,482
SYS-INT	31	368	3,388				3	26	305				34	394	3,693
MED-SCI	21	603	6,809				2	63	867				23	666	7,676
MARC	17	137	1,319				9	69	887				26	206	2,206
	202	3,303	31,214				28	286	3,248	1	7	69	231	3,596	34,532
POSTDOCTORAL INSTITUTIONAL (T32)															
PATH	17	106	1,723				1	8	151				18	114	1,874
GEN	22	120	1,936				1	5	91	1		3	24	125	2,031
CLIN P/T	15	53	936										15	53	936
TRAUMA	9	28	490				1	2	36				10	30	526
ANES	2	8	171				1	3	65				3	11	237
	65	315	5,256				4	18	344	1		3	70	333	5,603

NOTE: Discrepancies in totals due to rounding.

FY 1979 PBME CAREER AWARD AND RESEARCH GRANT SUPPORT
BY PROGRAM/ACTIVITY/GRANT TYPE
(Dollars in thousands)

PROGRAM AREA	ACTIVITY	TYPES 5 & 7 NO.	TYPES 5 & 7 DOLLARS	TYPES 2 & 9 NO.	TYPES 2 & 9 DOLLARS	TYPE 1 NO.	TYPE 1 DOLLARS	TYPE 3 NO.	TYPE 3 DOLLARS	TOTAL NO.	TOTAL DOLLARS
ANES	K04	4	146							4	146
	P50	1	907	1	716			1	53	3	1,676
	R01	8	498	5	362	10	750			23	1,610
	R23	10	367			4	131			14	498
		23	1,918	6	1,079	14	881	1	53	44	3,930
BIOENGR	K04	7	260					1	1	8	262
	K06	3	96							3	96
	P01	4	1,905	1	464			2	120	7	2,488
	P41					65	7,638			65	7,638
	P50	3	2,493	1	436					4	2,928
	R01	86	7,315	30	3,071	45	2,997	6	253	167	13,636
	R13					2	8			2	8
		103	12,068	32	3,971	112	10,643	9	374	256	27,056
RADIOL	K04	2	78							2	78
	P01	2	478							2	478
	P50	1	695							1	695
	R01	7	619	4	763	5	363			16	1,745
		12	1,870	4	763	5	363			21	2,996
TRAUMA	K04	2	73							2	73
	K06	1	33							1	33
	P01	1	282	3	970					4	1,252
	P50	6	2,669			1	457			7	3,126
	R01	35	3,123	5	541	16	1,167	1	50	57	4,881
	R23					6	256			6	256
		45	6,180	8	1,511	23	1,880	1	50	77	9,620
TOTALS (PBME)		183	22,036	50	7,324	154	13,767	11	477	398	43,603

NOTE: Discrepancies in totals due to rounding.

Report for the Office of the Assistant Director for Clinical Research

The major portion of the first year of the operation of this office was devoted to the Consensus Development Conference on Supportive Therapy in Burn Care which was held on November 10-11, 1978 at the Masur Auditorium, NIH. The conference was presented in cooperation with the American Burn Association and is part of a new effort by the NIH to bring together experts engaged in research and the practice of medicine to discuss controversial issues for the purpose of developing a consensus when possible.

The burn conference was devoted to discussions of five major issues: fluid resuscitation, metabolism, infection, smoke inhalation, and excision. Two ethical issues were also considered: under what conditions, if any, should early resuscitation not be performed in burns over 70% total body surface (TBS) and when would the administration of steroids be justified in response to smoke inhalation. The Institute was fortunate in having Dr. G. Thomas Shires, Professor and Chairman, Department of Surgery, Cornell Medical Center, chair the conference. Also, the panel members are world renowned for their work in burn medicine.

Participants from 33 states and seven foreign countries debated the issues for two days and with the participation of the entire audience came to a consensus on several of the issues. There was general agreement that total fluid replacement should occur within the first 24 hours postburn and range from two to four milliliters per kilogram per percent burn. All of the attendees with one exception felt that physicians have an ethical and moral responsibility to initiate therapy on all severely burned patients. There was general agreement that sepsis remains the leading cause of post-burn death, however, there were differing opinions as to the effectiveness of various forms of prophylaxis (i.e., debridement, use of wound coverings, barrier systems, and topical and systemic antibiotic therapy). Continual monitoring was considered essential on all patients who develop abnormal responses during the course of treatment. It was felt that more research in these areas is needed. Hypermetabolism was recognized as a life-threatening event in severely burned patients. Participants concurred that nutritional replacement is indicated in patients who have a greater than 20% TBS burn; pre-injury starvation; severe pulmonary, septic, or endocrine complication; or those who develop a 10% decrease in pre-burn weight during their hospital stay. When possible the oral route of nutritional replacement is preferred, supplemented by peripheral vein for administration of amino acid-fat emulsion solutions. Central vein is reserved for those patients who cannot be fed by the oral route. In adults, a formula of 25 Kcal/kg body weight plus 40 Kcal/% burn was acceptable with provisions for abnormalities. The requirements for children could not be formalized. With regard to smoke inhalation, it was agreed that the variety of insults which can occur require a more concise and universally acceptable classification. Also further research is needed to develop techniques which can more accurately define the damage from smoke inhalation. Pulmonary function tests, broncoscopy, and lung scans help to diagnose small airway obstruction but these techniques are not felt to be reliable for use during the critical phase of resuscitation. In analyzing the need for steroids, the participants unanimously agreed that their use is not indicated for any degree of smoke inhalation. There was no criticism of the need for cleansing and removal of debris in wounds, once the

patient's condition is stabilized. It was agreed that assessment of burn depth is critical. A consensus could not be reached on the issue of early excision. Autografts, allografts, and hemografts were all found to be useful. Among the issues regarding the use of these measures is the need to develop criteria for determining the status of the patient, the depth of the injury, and the appropriate degree of excision. Sequential excision should only be done in specialized burn centers and when the patient is hemodynamically stable. The participants concluded more research into the basic mechanisms is needed in many areas before consensus can be reached.

The conference was felt to be very successful and was used as a model by several NIH institutes in subsequent consensus development conferences. The technical summary of the meeting was widely distributed to the scientific community, interested lay people, and members of the press. As a result, numerous articles appeared in medical journals, lay magazines, and newspapers.

The full proceedings of the conference is in press and will appear in the Journal of Trauma this Fall (1979). Dissemination to the medical community of the conclusions reached at the conference will occur through the Journal distribution, NIGMS distribution, and National Library of Medicine information system. Periodically these computer summaries will be updated by former consensus participants who will cite references on the changes made. The first follow-up workshop will take place in the Spring 1980, approximately six months after the proceedings are published. The evaluation will have two major objectives: to determine whether the consensus recommendations have been disseminated and whether any new research in the five subject areas has affected the conclusions by the conferees.

As an example of new research, the Institute is aware of an important study in the area of fluid resuscitation that will probably influence a change in the approach to this issue. Following severe thermal injury there exists a negative nitrogen balance. This metabolic change requires resuscitation with considerable amounts of glucose and amino acids to provide energy in an attempt to bring about a metabolic balance. However the exact amounts of these exogenous substrates needed to provide sufficient energy and protein precursors for an optimum level of protein synthesis are felt to be an unsettled issue by Dr. John Burke, program director of the burn research center at Harvard and his associates. They have evidence that there is a maximal rate for infusion of glucose beyond which oxidation of glucose and protein synthesis does not take place. This is demonstrated by increased CO_2 production during infusion as well as large fat deposits in the liver at autopsy. The delivery of more than maximal quantities of these substances is made possible by easy access to the central venous route of infusion. The techniques used by this group of investigators give kinetic information on whole body glucose and amino acid metabolism. In nine burned patients with burns ranging from 20-61% TBS area, Dr. Burke has shown that at the highest rates of glucose infusion only approximately 40% of the glucose administered in oxidized, leaving 60% to be handled by other metabolic pathways. Although they have no direct evidence, they believe that possibly the metabolic fate of this unoxidized glucose is an ATP required conversion to fat and the production of CO_2 in excess of O_2 consumed. The presence of fat in the livers at autopsy is consistent with their concept. Further documentation in larger numbers of patients continues but in the

meantime, patients with elevated PCO_2, who have been receiving high rates of glucose infusions are being carefully evaluated for respiratory and hepatocellular abnormalities.

At the eleventh annual meeting of the American Burn Association (ABA) held on March 14-18, 1979, the Institute presented a two-hour panel discussion of the consensus conference on burns to its 2,000 attendees. The six panelists had also participated in the original conference. The Assistant Director for Clinical Research opened the discussion by defining the process of technology transfer and the role of consensus development conferences at the NIH. The five major areas of the conference were discussed by the panelists who also touched on the two ethical issues. The audience was in general agreement with the conclusions reached at the conference, expressed considerable interest in the consensus development process, and made numerous requests for copies of the existing summary and information on the proceeding publication.

The NIGMS is always well represented on the ABA speakers list by its grant supported scientists. At this meeting 26 papers were presented by NIGMS grantees and the following are brief abstracts of some of their work:

Dr. P. William Curreri, Director of the trauma-burn center at Cornell Medical Center is studying the in vitro growth of epithelial cells. One of the greatest deficits in the treatment of burn victims is the nonavailability of autologous skin in patients with massive injuries. Several attempts have been made to overcome this problem by growing epithelium in tissue culture. To date no method has given satisfactory results. Dr. Curreri has developed a new method to grow multilayered sheets of epithelial cells from single cell suspensions directly on plastic surfaces. The epidermis is separated using digestion by 0.25% trypsin for 12 hours at 4° C and is then mechanically stripped along the basement membrane. The epidermal cells are suspended in solution, seeded at a density of $5x10^5$ cells/ml into plastic dishes in a medium adjusted to a ph 5.8. The cells are grown in an atmosphere of 95% air and 5% CO_2. Cells adhere to plastic surfaces in 12-24 hours, start to grow in approximately 48 hours, and eventually form a confluent sheet. Once the cells reach confluence they start to "pile up." A multilayered structure is formed in 14-18 days. The surface area covered by the epithelial cell outgrowth is 10-15 times the size of the original skin explant. Such multilayered sheets when examined by electron microscopy are found to contain basal cells in the layer in contact with the plastic surface, and an additional 11-13 layers of cells resembling cells of the stratum spinosum, granulosa, and corneum of the normal skin. Since the basal cells are only loosely attached to the bottom of the tissue culture vessel it is possible to adhere sheets of such cells to pigskin or human skin. Utilizing this technique the basal cells are available to be placed directly onto a granulating wound. Clinical application is in process.

Several of the investigators at various NIGMS supported centers are examining the role of leukocytes in burn injury. Dr. Leonard C. Altman, at the trauma-burn center at the University of Washington in Seattle, has shown the importance of chemiluminescence (CL) of polymorphonuclear leukocytes (PMN) in patients with thermal injury. Following phagocytosis or activation with membrane activating agents, polymorphonuclear leukocytes produce a chemiluminescence or light emission which is oxygen-dependent and temporarily associated with

the activation of oxygen-dependent microbicidal mechanisms in the cell. Since thermally injured patients are unusually susceptible to infection, the CL of PMNs from these patients was examined. Peripheral blood PMNs were isolated on Ficoll-Hypaque gradients, suspended at $1x10^7$/ml in a standard electrolyte buffer and the light emitted by these cells measured using a liquid scintillation counter. Phagocyte stimulation and a nonphagocytic surface-activating agent were used. Studies were performed in 19 patients with an average burn size of 28.7%. Fifteen normal individuals served as controls. To evaluate the opsonic function of burn serum all studies were performed with normal as well as patient sera. In the absence of stimulation, PMN CL was similar in patients with burns of <20%. In contrast polymorphonuclear leukocyte CL was most depressed in patients with burns of 40% or greater. In addition burn sera was comparable to control sera in promoting CL of either control or burn PMNs. These results indicate that PMNs from patients with serious burns have decreased CL; that this defect is present whether a phagocytic or nonphagocytic membrane stimulator is used to activate PMNs; and that burn serum has normal opsonic function. Impaired PMN CL may reflect impaired cellular oxidative metabolism which could partially explain the increased susceptibility of burn patients to infection.

Defective bactericidal capacity of PMN leukocytes has been shown by Dr. Charles R. Baxter, principal investigator of a program project on burns at the University of Texas Southwestern Medical School, Dallas, to occur regularly in patients following major thermal trauma. Defective activation of the bactericidal mechanism either by the ingestion of bacteria or latex particles (NBT reduction) is accompanied by decreased oxygen consumption and a deficiency in NADH-NADPH oxidase activities. Dr. Baxter feels that the importance of these findings to oxygen-dependent mechanisms of bacterial kill should be reflected in the rate of formation of superoxide anion. The rate of leukocyte superoxide anion formation (determined by cytochrome C reduction) was serially measured in 16 burn patients (mean burn size 66%). These burn patients values were compared to the activity in normals, obstetrical patients (representing non-infected stress) and surgical infections (perirectal abscesses and appendicitis) to represent various types of *in vivo* stimulation of bactericidal activity. The results show that burn leukocytes did not respond to the stress of burn injury nor the additional stimulus of bacterial challenge. Conversely, they responded normally to *in vitro* stimulation suggesting inhibition of functional activity in the cells *in vivo*. The results of this study suggest that a significant defect exists in oxygen-dependent mechanisms for bacterial kill in leukocytes from burn patients and that inhibition in this activity is related to an inhibiting mechanism of unknown etiology which appears correctable by removal of the cells from the host environment.

Dr. Carol L. Miller, a project investigator in the trauma burn center at San Francisco California, has been able to characterize the suppressor cells which appear after burn injury. Immune suppression was examined repeatedly in 22 individuals (total of 240 assays) sustaining major thermal injury (30-85% full thickness burns). Sepsis developed in 14 patients and was fatal in seven. Severe inhalation injury was present in nine patients. Nine patients (six of whom died) developed severe sepsis associated with depressed response to phytohemagglutinin (PHA) five to eight days after injury. Concomitantly, addition of mononuclear leukocytes from these burn patients to normal mixed leukocyte

reactions (MLR) resulted in suppression of the high response of the normal combination. The mononuclear cell populations of these patients were depleted of thymus derived (T cell) lymphocytes by the sheet erythrocyte technique to examine the possibility that this suppressor cell was a T cell. These data demonstrate that suppression of the normal MLR by mononuclear leukocytes from patients with major thermal injury is reversed by T cell depletion. This is possibly the first direct evidence that T suppressor cells are activated after thermal injury, which may in part account for the immune depression seen after major burns.

Project REACH, the NIH contract awarded to the University of Washington for live telecasts via the Communications Technology Satellite on the NIGMS research program was successfully completed in February 1979. The initial satellite broadcasts took place in April 1978, between Bethesda, Maryland; Seattle, Washington; and Fairbanks, Alaska and a brief description of this event appeared in the annual report for 1978. These programs were video-taped with a series of tapes being excerpted for use by specialty groups (physicians, nurses, paramedics, dieticians, investigators in the field of burn research, and the lay public). In addition, four spot announcements were made for both public television and radio release. The general impact and acceptance of the broadcasts were assessed by a questionnaire sent to the medical and para-medical community of Fairbanks. About half of the community responded and all who did felt that the program was interesting and educational. The news media in Seattle showed considerable interest providing evening news coverage preceeding the broadcasts and follow-up reports on the progress of burn care and research at the NIGMS burn research center in Seattle.

The NIGMS Task Force on Technology Transfer and Assessment created in FY 1978 as a spin-off of the NIH Committee on Medical Applications of Research (OMAR) has provided valuable comments to the Assistant Director for Clinical Research on various trans NIH issues presented at the OMAR meetings which require institute input. In addition, the Task Force has analyzed the NIGMS role in the participation of consensus development conferences by examining the list and attending many of conferences presented by the various BID components of the NIH. It became more apparent that these conferences represent an interface with clinical application to which NIGMS has little to offer. However, it was felt that a consensus development conference in the area of liposomes, for example, showing the evolution of basic research into more applied investigations might be appropriate. The past few months have been spent in a thorough review of the national activities in the area of liposomes, a literature correlation and citation index search and consultations with several interested scientists. The Task Force concluded from its extensive review of the area that research on liposomes is advancing rapidly and that a conference in the Spring 1980 seems appropriate. Considerable support is provided by the NIH for the study of liposomes and within the NIGMS, three programs are involved in such activities. The idea was met with approval by the Director, NIGMS at the Task Force meeting in June 1979. Tentative plans include the assembling of a small planning group of consultants and NIGMS staff to meet by Fall 1979 to review the field, propose an agenda, and select tentative speakers.

As part of the worldwide observance of the United Nations International Year of the Child, the NIH developed a traveling scientific exhibit on the past,

present, and future research contributions which help children to grow to healthy adults. The exhibit, targeted to interest high school students, is travelling to 19 different cities during the year to appear at such assemblies as county fairs, medical and paramedical association meetings, the National PTA, and National Education Association. The NIGMS contributions to the exhibit were two panel sections on Birth Defects and Genetic Disorders and Burn Research and Treatment. Also the Institute provided copies of two publications, News and Features Portfolio on Burn Care Research and The New Human Genetics, for distribution at these gatherings.

In connection with the Fogarty International Center's US/USSR Individual Specialist Health Exchange Program, the NIGMS planned an itinerary for five Russian scientists, from the Central Research Institute of Reflex Therapy, to visit in November 1979 medical institutions in the U.S. which have active pain programs. The scientific mission was to familiarize the team with current American research trends in the area of pain and acupuncture research. Visits were made to a number of the NIGMS supported programs including the University of Washington Anesthesiology Center in Seattle and the Columbia University Medical Center's Anesthesiology Center in New York City. The scientists returned to Washington and met with the staff of the Fogarty International Center and the institute representatives involved in arranging the itinerary. They found the trip to be scientifically rewarding and extended an invitation to visit their Russian laboratories in an effort to generate collaborative programs.

CELLULAR AND MOLECULAR BASIS OF DISEASE PROGRAM

Objective

The Program's objective is to gain the fullest knowledge about human cells to assist in the prevention, treatment and cure of diseases of man. Obvious limitations of research on human cells *per se* prevent the most direct approach toward achieving this central goal. Consequently, the Program selects for support the most meritorious research on all manner of cell systems from the biotic world, within the accepted principle that living cells generally share fundamental biological and chemical properties. The supported research ranges from precise physical and mathematical approaches in the study of the finest chemical detail of enzyme catalyzed reactions in the cell to descriptive studies of newly discovered cell forms and their biological behavior as possible added model systems. Results from this broad range of research are reported continually in many scientific journals adding important new facts and general principles which contribute directly or indirectly to our expanded knowledge about both normal and abnormal function of human cells. A number of examples of the results of research sponsored by the Program during this fiscal year are presented in this report.

Program Scope

The diversity of research supported by the CMBD Program may be appreciated by some brief comments about the nature and function of cells. The living dynamic cell -- a highly organized array of ions and complex molecules -- is *the* fundamental biological unit of all life. The living cell holds the capacity for generating from rather simple exogenous sources all of its essential energy and enzymatic machinery for the most complicated of biochemical syntheses, varied movement and motility, and for self-regulated propagation. Cells form selective cell-cell associations of many kinds, continually display highly specific intercommunication over great distances (as free living microbes or within the human body), and interact with and adapt to all manner of environmental factors. Programmed by thousands of internal computer-like memory banks called genes, millions of offspring from a single egg cell are transformed into hundreds of groups of very specialized cells in an orderly fashion. This ordinary process occurs daily in the development of slime molds and humans, and all living forms in between.

It is the cell, or millions of them in higher organisms functioning in concert as special tissues or organs, that is the seat of photosynthesis, all growth, skeletal and heart muscle contractions, reception and interpretation of light waves, memory, action and reaction with environmental factors and signals, general behavior of all organisms, and even biological evolution.

Also, cells are the sites of infection and disease, and the ultimate disturbance of a relatively few cells in even the largest animal may result in death.

The variety of unicellular organisms and of cells from higher organism tissues available for study number in the thousands. Further, the intricate membrane surface and highly organized internal structure of each cell type, even the simplest cell, is of such complexity that groups of individual scientists may spend their professional lives studying the chemical structure and function of a single component or fraction of the cell. These cell components or compartments, often called cell organelles, in turn are collections of highly organized molecules ranging up to molecular weights in the millions.

Only a few examples of cell organelles currently being investigated in hundreds of laboratories over the nation with research grant support from the CMBD Program can be mentioned here. Mitochondria are discrete organelles in all cells, though highly variable in size and shape in different cell types and animal species. Mitochondria are the site of the major energy conversions and generation for all biochemical, electrical and mechanical activities of cells and their host organisms, including man. They are quite well-defined entities, microscopically and chemically. Each of the numerous mitochondria of each cell contain at least one hundred distinct types of enzymes. These are arranged in an intricately organized fashion on a convoluted membranous envelope which can be readily observed by electron microscopy. A special group of 20 or more coupled iron and copper enzymes, together with numerous associated chemical co-factors, in the mitochondria are referred to as the cytochrome or electron transport system. These enzymes function collectively in intimate association to generate high energy phosphate, ATP, for distribution and use throughout the cell for chemical synthesis, contraction, etc. Several major laboratories throughout the world conduct intense research on components of this enzyme system alone, many with support from the CMBD Program.

Microtubules, hollow, microscopic, rod shaped structures constructed from the assembly of rings of proteins called tubulin are ubiquitous cell organelles. Their role in cells seems to be multiple. They function as cytoskeletal structures contributing to cell form and shape. The contractile protein actomyosin may be attached to microtubules. The structure and arrangement of microtubules in parallel bundles, cross linked by a second protein, dynein, has been carefully worked out as the internal mechanism for constant motion of cilia and flagella, minute projections extending from the surfaces of many cells for locomotion. The fibrillar strands of the mitotic spindle on which chromosomes are pulled apart into each daughter cell in cell division are microtubules. Tubulin molecules readily assemble into microtubules and disassemble under controlled conditions. It is believed by some researchers that this may be the manner in which they move other cell components in cell division and possibly move selected protein active sites embedded in the cell surface membrane. Very recent evidence suggests that microtubules in the cell cytoplasm also may have a similar associated cross linking protein like those of flagella. Brain cells and all nerve cells (neurons) are rich in microtubule content. Their special role in the brain is not fully understood; however, there is considerable evidence that microtubules serve to transport other molecules along the extremely lengthy neurons of the body.

Each cell contains thousands of microsomes which are tiny spherical protein bodies, requiring very high magnification to be discerned by electron microscopy. They are attached to an extensive, convoluted inner cellular membrane called the endoplasmic reticulum (ER), and are the sites of all protein synthesis in the cell. Protein/enzyme synthesis in these ribosomes is under the direct control of the cell's genes through intermediate RNA molecules coded for the specific proteins to be manufactured. The ribosome itself is a complex chemical structure to be fully understood in the future, even though innumerable scientific papers have already reported a mass of data on its function and chemical detail. Quite recent findings from research supported by the CMBD Program have described the synthesis of proteins to be secreted from cells. Here, still another organelle, the Golgi apparatus, also a layered membrane structure, is involved. In a kind of assembly line fashion, prescribed amino acid chains, called signal peptides, from ribosomes pass into the ER membrane and are further coupled and folded into globular proteins. They are then transported to and modified in the Golgi and passed through membrane channels or vesicles to the cell surface for excretion into the extracellular environment, such as the blood stream or the lumen of an endocrine gland or the intestine. In microorganisms, this may be the mechanism and route for the excretion of digestive enzymes or of toxins.

A final example for mention is the outer plasma membrane of all cells which also may be considered as a distinct cell organelle. The plasma membrane is a transparent lipid bilayer, two molecule lengths in thickness of polar oriented fatty acid chains, with numerous kinds of protein molecules embedded throughout the film. Many of the specific membrane proteins are known to be highly mobile within the surface membrane. Some are known to form channels through the membrane for transport of ions such as Na^+, K^+, Ca^{++} as well as sugars, amino acids and other compounds into and out of the cell. Still other membrane proteins, many ligated with known glycosides, function as specific receptor sites for the attachment of hormones and other cell regulators emanating from distant cells in an entirely different organ of the human body. Cell surface antigens are yet other proteins and carbohydrates in the plasma membrane which become attachment points of antibodies. A cell's surface is also the site of attachment and penetration of viruses and other pathogens which attack cells. In all neurons, the plasma membrane functions to propagate the electrical current, via ion exchange, along its length.

CMBD grantees conduct research focused on the plasma membrane using approaches and techniques employed in immunology, enzymology, endocrinology, virology, bacteriology, chemistry and physics. Instrumental analyses of all sorts are employed in research on the plasma membrane and others of the cell organelles mentioned. Electron microscopy is used extensively in numerous laboratories in the study of membranes. The plasma membrane, only 60 to 100 angstroms thick, can be fractured by special freeze etch techniques into its inner and outer layers and photographed by the electron microscope. Sophisticated instruments for the measurement of nuclear magnetic resonance and circular dichroism, developed initially for wide use in chemistry are routinely used to study in situ the biochemistry and biophysics of cells and cell organelles. In the last few years, and with major expansion in the current year, CMBD grantees are now employing intense beam energies from high-energy physics

synchrotron machines to achieve more refined physical-chemical measurements of the intricacies of cellular molecules.

The general classes of compounds that make up the cell's organelles, the proteins, carbohydrates and lipids each comprise many molecular subsets. A major group of cellular and body fluid proteins are enzymes, and all such extra cellular enzymes are synthesized and secreted by cells. It is estimated that some 50,000 different enzymes have been identified or postulated for subsequent study, but only a fraction of these have been characterized in great detail. The complete chemical structure is known for a very few, but the precise mechanism of action in the fullest physical-chemical terms is known for none. To be sure, a great deal of valuable knowledge has been accumulated about enzymes and their specific catalytic role in metabolism, in synthetic reactions, in proteolysis, in other metabolic functions, and in cascading biochemical mechanisms such as blood coagulation and in molecular genetics. Much of this knowledge is now being applied clinically. Nevertheless, much remains to be learned of the precise chemical-physical mechanism of action of enzymes in order to fully understand cell function or cell dysfunction in many diseases. The same is true of many other classes of cellular molecules and associated trace metals and ions in terms of their structural, metabolic and regulatory role in the cell. Many, if not most of the separate functions and reactions of cells can now be attributed to specific classes or groups of compounds and ions within or attached to the surface of cells. We now recognize communication among molecules and information storage at many levels of sophistication in molecules that comprise cells and serve to communicate between cells. This is so in bacteria and in man.

Summary results from a variety of cell and molecular systems being investigated and the approaches used by CMBD grantees are included below.

PROGRAM ORGANIZATION: SUBPROGRAM AREAS

In order to effectively carry out its stated objectives, the program has been organized into separate subsections. The overall CMBD research program is administered by a Director and Deputy Director with support staff of nine professionals through a series of nine major subareas: (1) Cell Regulation, Differentiation, and Growth; (2) Cell Organization, Motility, and Division; (3) Membrane Structure and Function; (4) Enzyme Catalysis and Regulation; (5) Glycoconjugates; (6) Bioenergetics; (7) Structural Studies of Proteins and Related Model Systems; (8) Diffraction Analysis of Proteins and Related Model Systems; and (9) Theoretical Studies of Proteins and Related Model Systems. Intercommunication among staff members promotes valuable collaborative research efforts between scientists representing the various program subareas.

CELL REGULATION, DIFFERENTIATION, AND GROWTH
approximately 90 grants, $4,598,000

The Cell Regulation, Differentiation, and Growth Subprogram supports a spectrum of investigations on normal cell function that encompass many

active areas of cell biology. The research areas supported by the subprogram center on two major themes: cell differentiation and the cell cycle; these account for two-thirds of the subprogram's research investment. The other third supports a wide selection of research on diverse topics including cell adhesion and interaction, circadian rhythms, and the interaction of cell water with proteins and small molecules.

1. Cell Differentiation - 44 grants, $2,296,500

The largest group of research projects are those investigating the mechanisms of cell differentiation. Using a multiplicity of systems ranging from the cellular slime molds to the vertebrate cell in culture, from sea urchin eggs to multicellular algae, and from multicellular bacterial systems to nematode embryos, investigators are asking numerous questions concerning the process by which similar cells, during development, assume different functions. Subjects being investigated include: the role of external signals in the fertilization process; membrane and cell surface receptors; communication and interaction between cells; cellular compartmentalization; metabolic processes; and the mechanism of active hormones.

2. Cell Cycle and Control of Growth and Division - 25 grants, $1,216,300

The cell cycle of a growing cell is the period from its formation during fission from its mother cell to the time when it divides to form two daughter cells. Two separate classes of events occur in the normal cell cycle. One includes the physical events of mitosis and cell cleavage. The other includes the biochemical synthetic events which double the quantity of the organic components of the cell. This subarea is concerned primarily with the latter. Scientists are investigating the various control points within the cell cycle; regulation of synthesis of key components for initiation of genome segregation and cell division; control of gene expression during the cell cycle; and mechanisms of interaction of mitogens, hormones, polyamines, cyclic AMP, and external stimuli such as light in the control of cell growth and division. This problem has significance not only for understanding how embryonic development takes place, and how normal cell turnover and replacement is controlled in the adult, but also for an understanding of cell multiplication following tissue damage, immune response to infection, and the excessive production of abnormal cells which have escaped from normal regulatory controls, as in cancer.

3. Uncategorized - 21 grants, $ 1,086,200

The proposals which fall into the uncategorized section represent many diverse topics including some fields with only a small number of research-workers, new fields which are just beginning to develop and expand, and some fields where the less basic aspect is supported by other Institutes. Thus, the topics supported in this area include biochemical studies on silicone, mechanisms of cell fusion, regulation of leukocyte function, studies of cell adhesion and interaction, circadian rhythms, and the structure of water in cells.

CELL ORGANIZATION, MOTILITY, AND DIVISION
approximately 76 grants, $3,762,000

This group of grants involves studies of movement in cells ranging from gross cellular movements such as protoplasmic streaming to the movement of intracellular structures including both macro- and micromolecules. Movements of extracellular structures including flagella and cilia are included. Emphasis is on movement of and within non-muscle cells; however, a limited number of grants involving contractile mechanisms of smooth muscle cells are supported. Primarily, normal cell functions are being studied. Organisms used represent both procaryotic and eucaryotic cell systems. Investigations in this subprogram area draw upon a wide range of disciplines including biochemistry, immunology, physiology, electron microscopy, and cytochemistry at both the light and electron microscopic level.

The larger portion of the work supported here involves studies of cell structures such as microtubules and microfilaments, and the proteins associated with motile structures, including microtubular associated proteins, tubulin, myosin, paramyosin, meromyosin, actomyosin, actin, and various protein kinases. Motility studies involving control of shape associated with normal as well as transformed cells are supported here. Studies of the role of ions, principally Ca^{++} ions, and sulfhydryl groups in cell movement and cell division are included. Many of the projects emphasize the recurring theme of Ca^{++} involvement in regulation, whether it be the presence or absence of free Ca^{++} or the more recent function of calmodulin, the calcium dependent regulatory protein (CDRP). Cell division studies which center around the mitotic spindle, the movement of chromosomes and associated cellular proteins involved in the division process are included in this subprogram. Research focusing on the mitotic spindle include studies relating to the composition of this structure, its isolation, the movement of related structures with this apparatus, and its relationship to the cell membrane.

An area of grants which began to show growth in fiscal year 1978 and has continued through 1979 are those whose subject matter concern the interaction of cytoskeleton structures and the cellular membrane during movement and division. During 1979, this overall sub-program area has increased from 62 grants to 76 grants. At the annual meeting of the American Society for Cell Biology this past November, more than 30 presentations were made by scientists supported in this subprogram area. Several were invited symposia speakers. This growth is anticipated to continue in the approaching fiscal year.

MEMBRANE STRUCTURE AND FUNCTION
approximately 241 grants, $12,378,000

This sub-program deals with all aspects of the biology and chemistry of membranes. The emphasis is on the plasma membrane of cells, but a significant fraction of the effort is devoted to intracellular membranes. An overwhelming majority of the research is at the cellular or subcellular level. The principal investigators include cell biologists, chemists, biochemists, physiologists, biophysicists, and neurobiologists. This is a reflection of the present multidisciplinary approach to the study of cellular membranes and the emerging awareness of the central importance of membranes to the function of all cells. The research grants can be categorized in the following ways:

1. Active Transport - 44 grants, $2,621,900

It is apparent that since membranes separate the inside of the cell from the outside and are generally impermeable to most species of molecules, there must be specific transport mechanisms. Much of the research has focused on selected systems of broad importance in eukaryotes: sodium/potassium ATPase and amino acid transport. Some success has been achieved in the isolation of transport proteins and protein complexes. Reincorporation into reconstituted membrane systems is just beginning to show results. General advances in the manipulation of bacterial mutants and isolation of selected plasmids have been used to isolate and synthesize bacterial transport proteins. One emerging area is the cellular control of active transport. Inter- and intracellular signalling involving calcium and/or cyclic nucleotides is a rapidly growing subject of considerable interest.

2. Membrane Structure and Protein Composition - 91 grants, $7,062,700

This is the largest group of grants in this program area. This group contains highly sophisticated biophysical research. Questions of the organization and orientation of membrane proteins and multiunit complexes require elaborate instrumentation extending from purely microscopic observation to biochemical and biophysical approaches. Many investigators are now employing multipronged attacks, locating a protein by microscopic techniques and then isolating it for purification, sequencing, and reinsertion into lipid for diffraction studies.

3. Membrane Biogenesis - 25 grants, $1,654,400

Some of this work is solely biochemical, looking at the insertion of lipid or protein into membrane and the location,and pathway of timing of attachment of the carbohydrate moiety. The most exciting work is an extension of molecular biology which extends the already well-established studies of the synthesis of cytoplasmic proteins to those of membrane proteins.

The cycling of membrane, particularly between the plasma membrane and the cytoplasmic vesicles, ER, Golgi and lysosomes, is an expanding field. Only recently have some of the technical difficulties begun to be overcome by ingenious use of labels, clever selection of tissues and organisms, and development of ultrafast freezing techniques for electron microscopy.

4. Lipids and Lipid Properties - 38 grants, $2,335,800

Although NIGMS has long supported work on lipid monolayers and bilayers, it is only recently that there has been a literal explosion of interest in lipid chemistry, particularly in the area of physical chemistry. With improvements in nuclear magnetic resonance and electron spin resonance techniques, a great deal of effort has been expended in describing the microenvironment of proteins floating in the "lipid sea" of the membrane. There has been much excitement generated by the recent discoveries and measurements of the lateral mobility of proteins and lipids in the bilayer. Much work has been devoted to measuring the apparent viscosity of membrane lipids and determining the physicochemical state of the various lipid components of the membrane.

Recently, attention has been focused on mechanisms of lipid-lipid fusion, particularly with a view to possible use of artificial lipid vesicles to introduce enzymes and drugs into specific cells.

5. Passive Transport - 28 grants, $2,340,900

It is now clear that some intrinsic membrane proteins, singly or in combination, form channels through natural membranes. There has been some success isolating these complexes and incorporating them in artificial membranes. Other work has been devoted to the study of the kinetics and selectivity of channels. There has been some success at chemical modification of natural channels in vitro.

The field of synthetic ionophores has been growing rapidly. Some ionophores are being used as antibiotics and there is some evidence that naturally occurring insecticides are acting as ionophores. Excellent organic chemistry is being devoted to the synthesis of new ionophores and the modification of existing ones.

6. Other Membrane Enzymes - 13 grants, $850,900

These grants are devoted to membrane-bound enzymes, other than those involved in active transport. Although this is the smallest category of grants in the program, it includes some of the most interesting work in the entire membrane field. In some cases, such as in the study of membrane bound glycolytic enzymes, one of the main questions relates to the significance and advantage of a membrane bound location for these enzymes. In other cases, such as the study of NADH cytochrome B_5 reductase and cytochrome B_5 in artificial liposomes, the questions include the orientation of the individual enzymes and their relationship to each other during all stages of their active cycles. Some of the most elegant studies of tertiary protein structure and active site analysis have been carried out on the cytochrome B_5 system, which is involved in the metabolism of drugs.

ENZYME CATALYSIS AND REGULATION
approximately 224 grants, $11,261,800

Metabolism is a highly coordinated activity in which many sets of interrelated multienzyme systems participate in synthesizing and degrading the multitude of chemical substances involved in life processes. The central pathways of metabolism are remarkably similar in most forms of life and the reaction processes show an emerging pattern of fundamental mechanisms. Each metabolic step is mediated by protein catalysts known as enzymes. These molecules occur as multienzyme systems of varying degrees of relatedness, proximity, and interdependence. Together they control the occurrence and rate as well as the location and orientation of each metabolic event within the cell, and determine the relationship to alternative metabolic pathways.

This subprogram supports research on the nature of the active site of enzymes in relation to their catalytic activity; the regulation, control, and coordination of biochemical events within cells; the biochemical intermediates

produced in the catalytic process, and the production of cellular energy. Research approaches used range from fast reaction kinetic techniques and the synthesis of substrate analogs to the study of the stereochemistry of the intermediates formed in a given metabolic reaction. The entire program is directed at understanding the cell's basic catalytic machinery and how these mechanisms are regulated and fine tuned within living systems.

Research grants fall into seven main research areas and a small, eighth area containing other functions such as the support of conferences. The research areas are as follows:

1. Carbohydrate Metabolism [1/] - 59 grants, $3,791,200

Carbohydrate metabolism includes monosaccharides, oligosaccharides, polysaccharides, glycoproteins, and other glycoconjugates where the emphasis is on the carbohydrate portion of the macromolecule.

2. Lipid Metabolism - 21 grants, $1,440,800

Lipid metabolism includes simple or complex and neutral or charged lipids, steroids, and lipoproteins and lipopolysaccharides where the emphasis is on the lipid portion of the molecule.

3. Amino Acid Metabolism - 37 grants, $2,436,700

Amino acid metabolism includes the fixation of nitrogen ammonia and sulfur, and the metabolism of amino acids, polyamines, porphyrins and other nitrogen containing compounds.

4. Nucleic Acid Metabolism - 33 grants, $1,835,700

Nucleic acid metabolism includes purine, pyrimidine, nucleoside, monopolynucleotide metabolism.

5. Biological Oxidation/Reduction - 9 grants, $636,900

Biological oxidation/reduction includes electron transport proteins and oxidoreduction reactions that are not part of a major metabolic pathway, for example, super-oxide dismutase, catalase or other enzymes that use O_2, H_2O_2 or H_2 as an electron acceptor or donor.

6. Protein Peptide Metabolism - 17 grants, $1,288,600

Protein and peptide metabolism includes modification of the peptide backbone or covalent modification of individual amino acid residues within the peptide chain.

1/. This tabulation includes approximately 17 grants for $1,163,900 designated as Glycoconjugates and administered separately.

7. Conjugate Proteins and Enzymes - 44 grants, $3,014,600

Conjugate proteins and enzymes includes studies on the function and properties of the class of proteins containing prosthetic groups. They are defined by the chemical nature of their prosthetic groups, for example, phosphoproteins, metalloproteins and flavoproteins, rather than by their individual role in metabolism.

BIOENERGETICS
approximately 65 grants, $3,949,400

The Bioenergetics sub-program encompasses a collection of grants for the study of the mechanism of mitochondrial, chloroplast, and microbial oxidation and phosphorylation reactions. The goal of this program is a resolution and characterization of the components of the energy transducing apparatus and an understanding of their functional integration in cells. Grants for study of electron transport proteins and oxido-reduction reactions that are not part of a major metabolic pathway are also included in the program.

STRUCTURAL STUDIES OF PROTEINS AND RELATED MODEL SYSTEMS
approximately 172 grants - $ 9,107,600

This sub-program area represents physical and chemical studies on the structure of proteins, peptides, and synthetic polymers.

Almost one-half of the grants in this area deal with spectroscopic measurements of protein structure. Included are techniques such as nuclear and electron spin resonance, fluorescence, Mossbauer spectroscopy, circular dichroism and magnetic circular dichroism, and Raman spectroscopy.

A major area of study to which these techniques have been applied is the structure and function of metalloproteins. Because these proteins play a key role in the biochemistry of oxygen and in intermediary metabolism, they have been of central interest to biochemists. The availability of new spectroscopic methods, particularly EPR, Mossbauer, and magnetic circular dichroism, has revitalized the field. These techniques are highly sensitive to changes in, for example, the heme iron of cytochrome P450, so that details of events occuring during the enzymatic cycle can be monitored. This area of research is currently very active, and exciting new developments are anticipated.

Another major sub-area, comprising about 10 percent of the grants in this program, deals with determination of protein sequences. Such analyses provide data that is used for refinement of X-ray structures, evolution of structure-function relationships near an active or allosteric site, and determination of homologies between different proteins for the purpose of defining genetic and evolutionary relationships.

Many of the remaining grants in the program are mostly concerned with experimental or theoretical considerations of protein conformation. A major area of interest continues to be the forces controlling the folding

of protein and the acquisition of stable folded conformations. There are several groups approaching this problem using a variety of different techniques. Although progress has been slow, this is an extremely important area of research, and recent developments have been promising.

DIFFRACTION ANALYSIS of PROTEINS
approximately 55 grants - $3,660,700

This subprogram supports diffraction experiments on proteins and other macromolecules. During the last 25 years, X-ray crystallography has made a great contribution to our knowledge of the structure and function of proteins and other biological molecules. It is the only method that reveals precise atomic positions, and is thus crucial to other research in the field since the catalytic or functional behavior of all biological macromolecules is determined by the details of molecular structure. The research projects in this section can be classified according to molecular size and method:

1) Low Molecular Weight Structures - 8 grants, $337,200

X-ray crystallographic studies in this area are concentrated on small biomolecules, such as vitamins, complex lipids found in membranes, polypeptides, antibiotics, and carbohydrates found in a variety of biological systems. With smaller molecules, the crystallographic results are very accurate and can form the basis for stereochemical studies of larger systems. This is especially true for many of the refinement methods, where the macromolecule is constructed from small molecular components to fit the X-ray data obtained from the larger molecule.

2) Moderate Weight Proteins - 29 grants, $2,046,700

A decade ago few protein structures had been solved, and most of those were small, monomeric, extracellular proteins involved in hydrolysis. Today, a wide variety of protein structures have been determined. With advanced instrumentation and methodology, crystal size and quality is no longer the formidable barrier it once was. Proteins are now usually chosen for their biological or medical relevance. Many of the research projects in this group relate to such topics as enzyme mechanisms, energy transport, substrate binding and active site stereochemistry, protein folding, and protein interactions.

3) High Molecular Weight Structures - 12 grants, $845,900

This section of research projects is characterized by an assortment of both experimental techniques and biological systems. Most of the research utilizes X-ray crystallography, but also involves other methods including low angle diffraction, neutron and electron diffraction, and three dimensional reconstruction. The structure of large regulatory enzymes, enzyme complexes, and viruses can now be solved by X-ray crystallography. Such work would be impossible without recent advances in instrumentation. Knowledge of subunit interactions, quaternary structures, and macromolecular assemblies is needed for investigations of the function of enzyme complexes. A few projects are aimed at large oriented

macromolecular assemblies, such as membranes and polysaccharides. The primary tool for this research is low angle diffraction, with results centering on the location of components within a larger biological system.

4) Refinements and Methods - 6 grants, $317,100

Accurate atomic positions are essential for analyses of protein structure and function, such as substrate binding, active site stereochemistry, and intermolecular interactions. However, crystallographic data from macromolecules is seldom of sufficient quality to permit the determination of accurate atomic positions by traditional crystallographic means. The development of constrained refinements that utilize small biomolecular structures have greatly extended the accuracy of many protein structures. Much of the research in this section involves such protein refinements. Several other computational methods are also included.

THEORETICAL STUDIES of PROTEINS and RELATED MODELS
approximately 19 grants - $645,300

This small but important program area supports theoretical and mathematical research on a variety of biological topics. The vast complexity of living organisms and biological systems makes relevant theoretical studies difficult in conception, execution, and interpretation. In biology, as in all scientific fields, interesting theoretical calculations are often carried out on simplified and well-defined systems. Theoretical work is crucial to the overall biomedical research program, in that it provides the stimulus for many experiments. In addition, theoretical studies of simplified systems provide interpretations of experimental data from complex biological systems.

1) Structural and Physical Properties of Water and Aqueous Solutions - 11 grants, $407,800

This subprogram focuses primarily on water and its interaction with biological macromolecules. Modern techniques in the fields of statistical mechanics and molecular quantum mechanics are utilized by the researchers in this section of grants. Research on the structure, molecular dynamics, and physical properties of water and related research (e.g. on topics such as hydrophobic interactions, hydrogen bonds, and electrolytes) lead to an understanding of the vital role that water plays in determining the structure and biological function of proteins, nucleic acids, membranes, and other biological molecules.

2) Molecular Interactions and Potential Energy - 5 grants, $127,800

Various aspects of biomolecular interactions are examined through calculations of potential energy functions utilizing advanced methods of quantum chemistry. In most of these theoretical studies, results are linked to experimental data, and the resulting formulations are then useful in the interpretation and prediction of the molecular properties of proteins and other biomolecules. Problems examined include: protein folding, antibody-antigen interactions, drug-receptor interactions, electronic structures, and spectroscopic, thermal, and lattice vibration analyses.

3) Other - 3 grants, $68,600

This small group includes theoretical work on optical rotation theory, microwave effects on biological systems, and isotope effects in biochemistry.

RESEARCH HIGHLIGHTS

Presented below are examples of recent accomplishments of representative research projects being funded through the CMBD Program. These highlights reflect the broad range and kinds of activities currently being supported.

1. Cell Division Studied by Laser
GM 23445-02 (M. Berns) U.C., Irvine

Dr. Michael Berns of the University of California, Irvine, directs one of perhaps five laboratories in the world (the others are in Europe) which is equipped to perform sophisticated laser microsurgery on cells. He has applied this technology to the study of cell division with the support of GM 23445 [1/].

Historically, microbeam irradiation has been a technique which has contributed to the fields of developmental and cell biology. The technique has been used either to selectively alter small regions of living cells or embryos in order to study their function or it has been used analytically to microirradiate a part of a cell and record microfluorescence or microabsorbency. These experimental approaches, although they have been quite extensively applied over the past decades, have been limited by several constraints. The most important is that the radiation often employed has been classical non-coherent, polychromatic UV light. This type of radiation is often difficult to work with because it poses severe problems with cell survival, dosimetry, optics, and energy output.

The first laser microbeam device, using the ruby laser (red), was developed in 1962 by Bessis in Paris. The coherence of the beam, its monochromaticity, its high energy, and its other optical properties all suggested that it might be the ideal radiation source. Despite all the optical and energetic advantages of the ruby laser, however, it proved to be extremely limited in its application because it generated the wrong wave length for most biological studies. By 1969, the blue-green argon laser became available, and its usefulness in microbeam irradiation was quickly realized. The argon laser was subsequently outdated by the neodymium-YAG lasers and now by the dye lasers which provide all wave lengths from 200nm (UV) through the visible and to the infrared.

1/ He is also supported by GM 22754 for genetic studies on the consequences of chromosome deletion and by HL 15740 for studies on myocardial function using these techniques.

Mitosis has been one of the most intensively studied biological processes. Yet, despite extensive investigation, many of the most basic events of the mitotic process are either not clearly understood or are subject to controversy. Dr. Berns has chosen to study the role of the centriolar region in cell division and centrioles in mitosis. His work extends observations which have been previously described by Inoue,[2/] Borisy,[3/] and others.

The structure, segregation in cell division, and proximity to the mitotic apparatus of the centriole all suggest a possible role in cell division for this organelle. One role suggested has been as a site of nucleation of microtubules. In 1977, a previous study by Dr. Berns demonstrated that damage produced in the pericentriolar material of the prophase cells of kangaroo rat by argon ion laser microbeam irradiation was effective in blocking the anaphase movements of chromosomes. In order to determine whether the centriole itself plays a critically active role in the actual process of cell division, Dr. Berns irradiated the centriole region of prophase PTK_2 cells with a laser microbeam and allowed the cells to undergo mitosis. While subsequent electron microscopic analysis of cells demonstrated either the absence of centrioles or severely damaged centrioles at the irradiated poles, the cells were able to continue through mitosis normally[4/]. Thus, while the pericentriolar region seems to be necessary to normal cell division, the centriole itself does not appear to play an active role in the anaphase separation movements which are presumably mediated by microtubules.

In another study using light-activated, nucleic acid-binding drugs called psoralens, Dr. Berns presented evidence that RNA in the centriolar region is responsible for the formation of the spindle in dividing cells[5/]. Psoralens are planar molecules which intercalate into the nucleic acid helix between pyrimidines on opposite strands of the molecule. The absorption of near ultraviolet light by the intercalated psoralen molecule, produced by laser microbeam, may result in the formation of a covalent bond between the psoralen and the adjacent pyrimidines and subsequent inactivation of the nucleic acid. It is possible to choose specific psoralens with a high affinity for RNA and a low affinity for DNA. Treatment of cells with psoralen with a high RNA affinity, followed by activation of the psoralen in the centriolar region with a laser microbeam, resulted in a block in cell division. Examination of the cells by electron microscopy showed a decreased number of microtubules at the irradiated poles. Controls established that the effect (1) is

2/ NIGMS Annual Report FY 78, p.24.
3/ NIGMS Annual Report FY 77, p.21.
4/ Berns, M.W. and S.M. Richardson, 1977, Continuation of Mitosis After Selective Laser Microbeam Destruction of the Centriolar Region, J. Cell Biology. 75: 977-982.
5/ Peterson, S.P. and M.W. Berns, 1978, Evidence for Centriolar Region RNA Functioning in Spindle Formation in Dividing PTK_2 Cells, J. Cell Science, (in press).

not produced by the irradiation alone; (2) is not produced by a psoralen with a high affinity for DNA; and (3) is not a result of non-specific protein binding.

This study suggests that the abortion of mitosis in cells with psoralen reacted centriolar regions may be due to a modification of an RNA needed in the synthesis of proteins required in the formation of the mitotic spindle. The psoralen reaction in the centriolar region affects spindle formation but not anaphase chromosome movements when light activation of the psoralen is performed after spindle formation. The characteristics of psoralen binding and evidence from other investigators suggest that the RNA involved in spindle formation is not messenger RNA. The exact type of RNA and its precise function are the subject of further investigations.

2. Functional Model for Mitosis in Cell Lines
GM 23238-04,(Z.Cande), UC, Berkeley

Abnormalities in the process of cell division may lead to various forms of illness. For example, uncontrolled cell division is the basis of cancer and abnormal chromosome segregation is causal in several congenital malformations. Mitosis is thought of as a dynamic process comprised of various stages which allow for the duplication of the chromosomes and their separation during the division process. These stages include the condensation of chromosome structure, the breakdown of the nuclear envelope, the formation of the mitotic spindle, and the subsequent migration of the chromsomes on the spindle to its two poles.

Chromosomes are considered by many investigators to be passive with respect to their movement during mitosis. Several theoretical models have been proposed to study the force generation for chromosome movement, including the following: (1) force generation by changes in the length of microtubules which appear in the nucleus during metaphase as components of the mitotic spindle and considered to be responsible for chromosome movement during mitosis; (2) force generation involving dynein cross bridges of the microtubules and which have been shown to be a major factor in flagellar bending; and (3) force generation by an acto-myosin system interacting with microtubules. Actin and myosin, the proteins responsible for movement in striated muscle cells, have also been shown to be associated with spindle components in non-muscle cells.

NIGMS scientists whose research provides data supportive for each of the three theoretical models include Drs. Bajer, Borisy, Cande, Hepler, Inoué, Jenkins, Nicklas, Pollard, Salmon, Sanger, and Sherline. This highlight discusses the work of Dr. Z. Cande, whose research is providing a system to test the various models of chromosome movement described above.

Dr. Cande has developed a lysed mammalian cell system to study chromosome movement in vitro. A tissue culture cell line is utilized which after lysing with detergent, contains many intact organelles including a functional mitotic spindle that allows both chromosome-to-pole movements and spindle elongation at 40% to 70% the rates found in whole cells. This permeabilized cell model

system allows for the manipulations of chromosome movement, i.e., stopping and restarting with specific chemical inhibitors and additives such as adenosine triphosphate (ATP) and magnesium (Mg^{++}).

Using polarized optics and electron microscopy, Dr. Cande is able to monitor the stability of the spindle structure during various experiments. By investigating various physiological parameters of the spindle, interesting useful data has been forthcoming from Dr. Cande's laboratory. For example, lysed cell spindles will continue to move chromosomes in the absence of ATP, but at a lower rate than in non-lysed cells. Added ATP significantly speeds the rate of movement. The role of ATP as an energy source for the cell mitosis processes was examined by uncoupling oxidative phosphorylation through the use of inhibitors at the time of lysis. Time studies showed that even after 4 minutes in the absence of ATP by this method, spindle function can be restored by the addition of fresh ATP. The specificity of this reaction was checked by using two nonhydrolyzable nucleotide analogues of ATP which were not able to reverse the inhibition as did ATP. These experiments provide direct evidence that mitosis is dependent on an enzymatic process, namely ATP hydrolysis. This represents a commonality between mitosis, flagellar beat and muscle contraction. Magnesium is also required in this system as demonstrated by the use of a chelating agent followed by the addition of Mg^{++} in experiments which restored chromosome movement.

The Dynein-ATP complex has been shown to have a key functional role in beating movement of flagellar and cilia. Vanadate at micromolar levels is an inhibitor of dynein ATPase activity and beat of demembranated flagella. Myosin ATPase activity is not affected at these low levels. Using vanadate in the lysed cell system described above, it has been shown to reversibly inhibit chromosome separation and spindle elongation at concentrations which do not block myosin activated myofibril contraction. The results of these experiments provides indirect evidence for a role for dynein in chromosome movement and in the maintenance of spindle structure. This is significant because of the demonstrated contractile role of dynein cross bridges in the movement of flagella and cilia. Since movement of flagella beat has been elucidated, analogies of this sort, albeit indirect, are helpful in understanding chromosome movement during cell division. Antisera against cytoplasmic myosin employed in the lysed cell system do not retard anaphase. Dr. Cande suggests that this finding, along with the vanadate data, indicates that myosin is probably not the sole or major enzyme responsible for chromosome movement, unless the presumptive spindle myosin differs from cytoplasmic myosin. He suggests that it is possible for two enzyme systems, dynein and myosin, to operate in parallel to move chromosomes.

Another interesting finding is that exogenous tubulin retards chromosome movement. If tubulin, the protein sub-unit of microtubules, is added to the lysis medium at concentrations above a critical minimum concentration of 0.8 mg/ml, chromosome separation and spindle elongation rates are retarded by 33%. Higher concentrations of tubulin retard the distance of chromosome-to-pole movements by 45 percent and spindle elongation by 25 percent. Dr. Cande's group believes that this data supports aspects of the hypothesis of microtubule polymerization-depolymerization as a mechanism for chromosome movement. However, the data does not support microtubule polymerization as a force-generating process for spindle elongation.

3. Cell Development Studies in Physarum Polycephalum
GM 18538-08 (Kuehn) New Mexico State University, Las Cruces

Polyamines and their biosynthetic enzymes are ubiquitous. In some microorganisms they have been definitely shown to be required for growth. The concentrations of polyamines and their biosynthetic enzymes increase when the growth rate increases in a number of normal and neoplastic cell systems. (See, for instance, reference number 3.) These increases usually precede or are simultaneous with increases in RNA, DNA, and overall (or other) protein levels. Such observations have led to speculation that polyamines are involved in regulating growth; however, no specific mechanism has been firmly established for the action of polyamines, in vivo.

In two recent papers (1,2) Dr. Glen Kuehn and his coworkers report an exciting series of observations which more strongly and specifically link the polyamines to regulation of ribosomal RNA synthesis. First, polyamines were found to stimulate selectively the phosphorylation of two non-histone acidic proteins in isolated nucleoli from Physarum polycephalum. Second, they have demonstrated that one of these phosphoproteins, of 70,000 molecular weight, stimulates rRNA synthesis 5-fold, and binds with high affinity and specificity to the palindromic ribosomal DNA which contains the coding sequences for three ribosomal RNA's.

Intact isolated nuclei and nucleoli incorporated inorganic ^{32}P into the phenol-soluble acidic nuclear proteins in the presence of glucose, Mg^{++}, and ATP. Various mixtures of different polyamines at 1 nM total amine concentration stimulated phosphorylation of the nuclear proteins compared to controls lacking polyamines. The magnitude of the enhanced phosphorylation ranged from 6-fold to 30-fold depending on the combination of polyamines used and the given nuclear preparation. Although Mg^{++} is required for the reaction, it could not substitute for polyamines in effecting the stimulation. Fractionation of the phosphorylated proteins by polyacrylamide gel electrophoresis revealed that different polypeptides were phosphorlated by nuclei in the presence versus the absence of the polyamines. In the presence of a specific mixture of polyamines, enhanced phosphorylation occurred in four major nuclear proteins of molecular weights 14,000, 27,000, 52,000 and 70,000. In the absence of the polyamines, three major proteins were phosphorylated with molecular weights

1/ Atmar, V.J., Daniels, G.R., and Kuehn, G.D. (1978), Polyamine Stimulation of Phosphorylation of Non Histone Acidic Protein in Nuclei and Nucleoli from Physarum polycephalum, Eur. J. Biochem. 90: 29-37.

2/ Kuehn, G.D., Affolter, H., Atmar, V.J., Seebeck, T., Gukler, U. and Brown, R. (1979), Polyamine-mediated Phosphorylation of a Nucleolar Protein from Physarum polycephalum that Stimulates rRNA Synthesis, PNAS, 76: 2541-2545.

3/ Clark, J.L. and Duffy, P. (1976), Polyamine Metabolism, RNA Synthesis, and Proliferation in Density-Inhibited 3T3 Cells, Arch. Biochem. Biophys. 172: 551-557. GM 21854.

of 17,500, 21,000 and 25,000. The two proteins whose phosphorylation was most stimulated by polyamines (52K and 70K) were found to be of nucleolar origin.

Dr. Kuehn and his colleagues purified the 70,000 molecular weight protein after its polyamine-stimulated phosphorylation. The rRNA synthesis by RNA polymerase I, within a nucleolar ribosomal deoxyribonucleoprotein complex (a minichromosome), was stimulated 5-fold by the purified 70,000 phosphoprotein. It was shown by hybridization experiments that more than 70% of the labelled RNA synthesized by this minichromosome system was complementary to those sequences of rDNA that coded for 19S and 26S rRNA.

Since the association of the phosphoprotein with the rDNA complex suggested possible DNA-binding properties, Dr. Kuehn tested its binding both to ribosomal DNA and to several restriction endonuclease fragments of this DNA. It bound with high affinity and specificity to the palindromic ribosomal DNA which contained two coding sequences for 5.8S, 19S, and 26S rRNA respectively. It also bound to three restriction endonuclease fragments of rDNA which had in common the symmetry axis for the rDNA palindrome. Further, this binding region is distally removed from the known coding regions for 19S and 26S rRNA.

In arguing for a regulatory function of the acidic, non-histone chromosomal phosphoprotein, Dr. Kuehn points out that the phosphoprotein stimulated transcription of the ribosomal genes within the nucleolar rDNP complex; it bound with high affinitiy and specificity to a defined region of rDNA; and neither property was retained after dephosphorylation of the protein. However, it is not known at this time whether binding of the phosphoprotein to rDNA is a prerequisite for stimulating transcription within the rDNP complex. If the binding of the phosphoprotein to rDNA is indeed required for stimulating transcription of these gene sequences, there are the interesting questions of how this is achieved over such a large intranucleotide distance (10K base pairs) and what role the palindromes may have in the process.

4. Enzymes of Aromatic Oxidations from Microorganisms
GM 20172-05 (Ribbons) University of Miami

The long range goal of Dr. Ribbons' research program has been to understand the biochemical and genetic basis for the events that occur during the evolution of bacteria and which allow mutant organisms to grow on novel compounds that the parent strains cannot utilize. This investigator has had a continuing interest in the relationship of the specificity of catabolic pathways and their control to the nutritional characteristics of bacteria in the utilization of aromatic compounds.

His approach is to compare the specificity, regulation, structure and mechanism of enzymes of the pathways, as they occur in the parent strains, with those found in mutant descendants, which are now distinguished by the acquisition of new nutritional capabilities. A study of the specific events that allow the selection of mutants to grow with novel compounds should provide a

rationale for the directed evolution of microbial strains with enzyme complements (or catabolic pathways) for the catalysis of reactions potentially useful to man.

The efforts of this group have recently elucidated the orcinol and resorcinol catabolic pathways in two strains of Pseudomonas putida. They have found that whereas the first organism (strain 01) can acquire the ability to grow with resorcinol by constitutive expression of enzymes of the orcinol pathways, the second (strain ORC), possesses a mechanistically and genetically distinct set of enzymes. Orcinol hydroxylases from both strains and a resorcinol hydoxylase from strain 02 have been purified to homogeneity and several of their properties compared. These functionally homologous flavoproteins from strain 02 are remarkably similar in gross physico-chemical properties and amino acid composition, but very distinct in their catalytic and serological properties. Acetylpyruvate hydrolase, a novel hydrolytic enzyme in the orcinol pathway, has been obtained in pure form from strain 01 and some of its properties have been examined. These studies and their relationship to the observations of others indicate: (a) for catabolism of resorcinylic compounds the introduction of a hydroxyl group is an essential prerequisite as a preparation for oxygenative-ring cleavage; (b) the flavoprotein hydroxylases are very versatile catalysts and probably the most prevalent group of enzymes in microorganisms that catalyze monohydroxylation of the aromatic nucleus. Dr. Ribbons' group has also elucidated the reaction pathway for the compound p-cymene used by a mutant strain of Pseudomonas putida designated strain PL. They found the p-cymene pathway is very non-specific as shown by the large number of analogs that are readily metabolized by p-cymene-induced cells. It is also clear that very many analogs are good inducers of some (or all) of the enzymes of the pathway. Several acquisitive mutants have been obtained, that possess the capability of growing with certain analogs as sole carbon source which is impossible for the wild-type parent strain. Genetic and physical evidence has been obtained to demonstrate that the genes coding for the p-cymene pathway in strain PL are present on a self-transmissible plasmid.

The research on the oxidation of the halogenated derivatives of p-cymene by strain PL and the work elucidating the role of the "2,3-Dihydroxybenzoate" metabolic pathway in the degradation of the aromated nucleus of natural and synthetic compounds has important implications. It will provide methodology for the directed evolution of new catabolic activities by microorganisms and will provide information on the metabolic potential of soil, water and sewage microorganisms.

By mutation and selection of strains of bacteria with catabolic enzymes of new and different specificities, the way is opened for the provision and use of designed enzymes to catalyze specific chemical transformations not readily achieved by conventional chemical procedures. These new strains or their extracts might therefore by used to: (a) improve sewage and effluent disposal systems; (b) produce hydroxylated metabolites of the p-cymene pathways with the anti-pyretic and the anti-inflammatory properties of salicylate and its dervatives (e.g., aspirin), some of which (e.g.,

2,3-Dihydroxybenzoate) have reduced gastric irritancy; (c) indicate chemicals which might undergo biodegradation and those that are likely to provide toxicological problems to the natural environment by lack of degradation or degradation to a product more hazardous than that one introduced.

5. Coenzyme Analogues as Probes of Flavoenzyme Catalysis
GM 21643-05 (Walsh) Massachusetts Institute of Technology

Organisms contain a large variety of enzymes which catalyze the oxidation or reduction of organic and inorganic substrates. Most enzymes catalyzing redox (oxidation/reduction) transformations can be divided into two types according to the cofactor they require to mediate electron transfer. They are: (1) Those which use transition metal ions as conduits for electron flow; and (2) Those which use conjugated organic coenzymes as intermediate electron carriers.

The two major redox coenzymes in simple enzymatic dehydrogenations are the nicotinamide-based coenzymes (NAD/NADH and NADP/NADPH) and the flavin coenzymes. The NAD and NADP coenzymes have been more intensively studied for mechanisms and substantial information on their mode of action has been obtained. The flavin coenzymes can accomplish the same functions as dehydrogenases cofactors but are considerably more versatile in their chemistry and consequently in their biological roles. For every type of substrate dehydrogenation that is mediated by an NAD-using dehydrogenase, one can find from another biological source, a flavin-linked enzyme carrying out the same transformation (e.g., lactate or glutamate dehydrogenases). The ubiquity of flavin is matched by its functional versatility as a redox cofactor, which probably exceeds by far that of any other coenzyme or prosthetic group. Thus, through binding and reaction specificity exerted by its respective apoenzyme, flavin becomes, inter alia, a dehydrogenase, an oxidase, an hydroxylase, an electron carrier or a light emitter. It is now a truism to state that virtually every electron that is transported in the biosphere has spent at least a millisecond or so somewhere in this coenzyme's nucleus.

Over the past decade the NIGMS has sponsored a very active program of research on the flavoproteins. A CRISP system printout with a flavoprotein enzymes search title indicates that of all NIH sponsored projects in this field NIGMS sponsors the critical nucleus (about 36%). The remainder are scattered over many other Institutes with the flavoprotein work frequently forming a component of a larger program project. The investigators funded by NIGMS in this area constitute a "who's who" of leaders in this field. Dr. Christopher Walsh, MIT, is one of these leaders. The focus and scope of Dr. Walsh's research effort has been to analyze the chemical properties of the isoalloxazine ring system of the riboflavin-derived coenzyme, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), by the use of flavin analogues in an attempt to understand how these ubiquitous cofactors function.

The Walsh laboratory has prepared a varety of endo- and exo-cyclically substituted riboflavin analogues including 1,5-deazaflavins, 3-deazaflavins, and a number of 8-substituted flavins. They analyzed the electronic spectra, pKa values and other structural and chemical properties with respect to

their preferred tautomeric and resonance forms. They are using this knowledge and these compounds to examine important physiological redox phenomenon of enzymes and are obtaining results with biomedical implications.

All the endo- and exo-cyclically substituted analogues were tested by this group as coccidiostats, i.e., antagonists against parasitic infections of chickens. The 5-deazaflavins were the most potent, however, in their present form their therapeutic index, vis a vis toxicity, is not high enough for use. They also showed that dihydroflavins cause a rapid and complete reductive mobilization of iron from overloaded ferritin, the major iron storage protein. They quantitated the release rate with flavin analogues and found the stronger the reductant the faster the release rate suggesting this might be a useful tool for iron overload studies. This condition occurs in several diseases requiring chronic transfusions such as Cooley's anemia and thalassemia.

6. Ion Gradients and Energy Coupling in Bacteria
GM 24195-02 (Maloney) Johns Hopkins University

For years now, many details of the breakdown and oxidation of foodstuffs have been known, but the precise mechanism whereby the energy released in this oxidation is transformed into a usable form in the cell has resisted all attempts at description. The major inroad in understanding this transformation, be it generation of ATP or osmotic work, came from Peter Mitchell (see previous comments on Chemiosmotic Theory).

The critical realizations of Mitchell were: (1) that a membrane could allow a vectorial character for membrane-associated reactions, thus allowing (2) a special segregation of reactants and products, in particular the creation of a proton gradient; (3) that a reversible, proton-translocating ATPase/ATP synthetase existed which would utilize a proton gradient; (4) that the elusive coupling of oxidation to phosphorylation is the circulation of protons; and finally (5) that the power in the proton gradient can be delivered to a variety of processes within the membrane which are not directly coupled to the enzymes producing the gradient.

It has generally been assumed that, because the vectorial flow of protons in one direction (outward in mitochondria) is driven by ATP hydrolysis, the flow in the opposite direction is coupled to ATP synthesis. The actual experimental confirmation of that assumption has depended on the use of various inhibitors which either facilitate or stop the flow of protons along the electrochemical gradient. In microorganisms, such an assumption might also be amenable to testing using mutual organisms.

Dr. Peter Maloney at Johns Hopkins University has utilized a mutant of E. coli lacking a functional ATP-synthetic enzyme complex to make a direct demonstration that ATP-synthesis is coupled to proton entry, as required by the chemiosmotic theory. The enzyme complex, BF_oF_1, consists of bacterial membrane fragments (BF_o) to which the bacterial ATPase

enzyme (F_1) is attached. E coli strain AN180 (wild type) and AN120 (uncA), lacking a functional ATPase, were chosen because they have the same passive permeability to protons.

Dr. Maloney utilized two basic experiments. In the first, he lowered the external pH from 8 to 3.5, creating a potential across the membrane and causing protons to flow into the vesicles. Dr. Maloney found that ATP was synthesized in the wild type, but not in the mutant. ATP synthesis was not apparent when the proton conductor FCCP was present to allow proton entry to bypass the F_1 enzyme. Permanent anions (such as nitrate or chloride) were required for ATP synthesis. The poorly permeant anion sulfate did not support ATP synthesis. In a second set of experiments, Dr. Maloney sought to demonstrate that the ATP synthesis is linked to the entry of acid equivalents. ^{14}C-salicylic acid, whose concentration in the vesicles is pH dependent, was used to measure the flux of protons. When the pH jump is imposed by sulfuric acid, the internal pH change in wild type and mutant BF_oF_1 preparations are the same, reflecting the similar passive permeability to protons of the two strains. This conclusion is derived from the fact sulfate is only poorly permeable at acid pH and will therefore not support proton entry. When the jump is imposed by hydrochloric or nitric acid, internal pH falls, the drop being greater in the wild type than in the mutant cell vesicles. This increased entry is attributed to the entry through BF_oF_1 of protons during synthesis of ATP and is consistent with similar pH jump experiments Dr. Maloney has conducted with S. lactis vesicles utilizing inhibitors instead of mutants. The stoichiometry is 5 protons/ATP (or less depending on assumption concerning internal buffering power and ATP-consuming reactions).

Dr. Maloney's results provide elegantly simple confirmation of a basic element of the chemiosmotic theory and demonstrate its applicability to bacterial systems as well as to mitochondria and chloroplasts. Work is now in progress in many labs demonstrating the applicability of the chemiosmotic theory to ion transport and energy conservation reactions in animal cells. Future highlights will focus on some of the successes of the chemiosmotic theory in rationalizing various experimental observations.

7. Mitochondrial Membrane Metabolic Role of Cytochrome C
GM 19121-07 (Margoliash), Northwestern University

The respiratory chain of mitochondria consists of a set of proteins and cofactors located in the inner membrane of the mitochondria. The chain transports electrons from substrates to oxygen and in the process generates an energy pool which in one form or another meets all the energy requirements of the organism. The terminal portion of the respiratory chain consists of a small protein and of a large protein complex. The former is cytochrome c, a heme protein of 12,400 molecular weight and the only respiratory chain protein not fully anchored in the inner membrane, being free to move about in the space btween the inner and outer mitochondrial membranes. Its function is to transport electrons to the last member of the respiratory chain, the large assembly of 7 different

varieties of protein chains, hemes and copper atoms, with an overall molecular weight of about 250,000, known as cytochrome c oxidase. It is firmly embedded in the inner mitochondrial membrane. With the electrons obtained from cytochrome c it reduces to water the molecular oxygen delivered from the lungs to the tissues, thus carrying out the final reaction of tissue respiration.

New understanding of the functional organization of the terminal segment of the respiratory chain has come from the laboratory of Dr. E. Margoliash with support from NIGMS. Two new approaches have been responsible for these results. The first is the use of a new polarographic method capable of measuring the interaction of extremely low concentrations of cytochrome c with cytochrome oxidase. This method has permitted investigation of very high affinity reaction representing the primordial function of cytochrome c, namely the transport of reducing equivalents from cytochrome c reductase, the preceding segment of the respiratory chain, to cytochrome c oxidase. In performing this phase of its function, cytochrome c does not need to leave the surface of the inner mitochondrial membrane but moves on it, remaining oriented so that the edge of its heme, on the front surface of the molecule at which electron transfer occurs, is against the membrane and therefore in direct contact with the membrane proteins. This favorable orientation is maintained by the remarkably asymmetric distribution of charged amino acids on the surface of the cytochrome c molecule. This distribution develops an electrostatic dipole moment with the positive end of the dipole axis at the edge of the heme held against the negatively charged membrane. Cytochrome c is thus constructed to perform as a particularly efficient mobile electron transporter within the respiratory chain gap between the reductase and the oxidase enzyme systems, both of which are embedded in the inner mitochondrial membrane.

Dr. Margoliash has further demonstrated in studies of a variety of primate cytochromes reacting with primate and non-primate oxidases that the initial binding of cytochrome c is followed by a conformational change on the front surface of the cytochrome. This new conformation leads to formation of a productive complex which allows electron transfer beween the donor and acceptor centers. The rate of this conformational change is regulated by the strength of two electrostatic interactions between amino acid side chains on the surface of cytochrome c.

Study of the nature and location of these electrostatic interactions, both on the surface of cytochrome c and between cytochrome c and the oxidase has benefited from the second major advance in Dr. Margoliash's laboratory. Highly purified chemical derivatives of a large portion of the 19 lysine residues on the surface of cytochrome c have been prepared. These modifications, by causing local changes in charge and in surface structure, make it possible to map the surface areas on cytochrome c at which the protein reacts with its various electron exchange partners. These so-called interaction domains have turned out to be nearly the same for cytochrome c oxidase, cytochrome c reductase, yeast cytochrome c peroxidase, sulfite oxidase and cytochrome b_5. All are at the front and upper surfaces of the molecule in a region that includes a good deal, if not all, of the

exposed edge of the heme prosthetic group. This circumstance makes it nearly certain that the electron is indeed both taken up and given off by the heme edge. Since these interaction domains all cover the same section of the molecular surface, to obtain an electron from one of these reaction partners and deliver it to another, cytochrome c must move from one to the other. It is the multiple forms of mobility the protein must display to carry out its several physiological functions that explain the need for the highly asymmetric distribution of charges on the molecule, for the high level of affinity with which it reacts with cytochrome oxidase, and for its confinement to the narrow, inter-membrane space of mitochondria. Without such restriction, cytochrome c could diffuse throughout the entire cell, locate itself on the many negatively charged surfaces other than the inner mitochondrial membrane, and cut down its effective concentration at the respiratory chain to the point that the system would function much too slowly to satisfy the energy requirements of the organism.

8. New Developments in the Application of NMR to Complex Biological Systems GM 16552-11, (Waugh) MIT; GM 23289-03 (Griffin) MIT; GM 24266-01 (Opella) U. Pennsylvania

Except for x-ray crystallography, nuclear magnetic resonance (NMR) appears to offer the richest source of detailed structural information about molecular systems. Furthermore, NMR has the potential of providing a dynamic picture of molecular events, as opposed to the static picture obtained from crystallographic studies. In view of this promise, it must be admitted that NMR studies of biologically important systems have been somewhat disappointing. The clear structural details observable in the NMR spectra of small molecules have, with some exceptions, not been obtained with macromolecules. The proton resonances, which are the most common resonances observed in the application of NMR, are simply too numerous to be adequately resolved even in small proteins. In addition, the motional narrowing which sharpens the spectral lines of smaller proteins and other small molecules is non-existent or incomplete in nonglobular monomeric proteins and supramolecular assemblies such as membranes. In many ways, the spectra of such structures more closely approximate the spectra of solids rather than of relatively dilute solubilized systems. The signals from solids are typically broad and rather featureless, making it almost impossible to extract detailed structural information. The development of experimental methods for analysis of spectra which are typical of solids has therefore been the goal of several research groups. Perhaps the most successful of these groups has been that of Dr. John Waugh, at MIT. This highlight will describe some work by Dr. Waugh and two of his former students, Dr. Griffin and Dr. Opella.

Dr. John Waugh is a physical chemist who, by his own account, is "unqualified by talent or training" for research oriented directly toward problems in biology. Although Dr. Waugh undoubtedly underestimates his abilities, it is true that his major contributions are in the realm of theory and instrumentation. He approached the problem of solid-state spectra by asking fundamental questions about the "averaging" process found in NMR specspectra of molecules in solution. The fundamental difference between solution-state and solid-state NMR is that in the former the rapid rotation of

molecules "averageout" the anisotropic interactions between nuclear spins. Since the -carbon backbone of native proteins is sterically restricted, it is a reasonable approximation to treat these carbons as if no internal motions are present and their effective mobility is that of the entire protein. Clearly, the motions of very large, or very asymmetric, proteins is quite slow, so that motional averaging is incomplete. The studies of Waugh and his colleagues have resulted in what is called "coherent averaging theory". This describes those cases where the spin interactions are regularly periodic rather than randomly varying, as in liquids. Such periodic motions can be externally imposed, either by rotation of the sample or by externally applied magnetic fields, or both. Dr. Waugh has developed several experimental approaches, of which the most widely applied is the method described as proton enhanced nuclear induction spectroscopy (described below). Dr. Waugh is now concentrating on another approach, which involves spinning the sample at the so-called "magic angle". It appears that such rotation can produce similar spectra from incompletely mobile systems as is found in liquids. Despite Dr. Waugh's reservations about his capabilities, his most recent progress report indicates that he is initiating studies on systems of more specifically biologic interest, such as membranes and DNA.

Dr. Waugh's methods have been applied to biologically relevant systems by two of his former students. One of these, Dr. Robert G. Griffin, has focused on questions involving the structure and dynamics of phospholipid bilayers. The approach is primarily that of proton enhanced nuclear induction spectroscopy, mentioned above. This technique involves cross-polarization of "rare" spins, e.g. ^{13}C, ^{15}N, ^{2}H, or ^{31}P, by first irradiating the abundant spin species, for example protons, and allowing transfer of spins between the abundant protons and the dilute spin system to occur. The signal of the dilute spin system is then observed with decoupling of the proton resonances. It is possible by this approach to observe chemical shift anisotropies, i.e., the directionality of the magnetic interactions. Using this technique, Dr. Griffin has been able to analyze the head group orientation in ^{31}P-labelled oriented bilayers of dipalmitoylphosphatidylcholine. It has been possible to follow quite precisely the degrees of disorientation introduced by cholesterol in the bilayer. More recent studies indicate that high resolution spectra from such bilayers can be obtained by proton enhanced resonance plus spinning the sample. Work on this is now proceeding.

Perhaps the most ambitious study now underway is that conducted by Dr. Stanley Opella, another of Dr. Waugh's former students. Dr. Opella is attempting to follow the interaction of DNA and the coat protein, and gene 5 protein, of bacteriophage fd. ^{2}H and ^{13}C- labelled histidine and tyrosine will be prepared and incorporated into viral protein. Since these are relatively few in number, and thought to be involved in protein-DNA interactions, the NMR methods described above may allow analysis of the nature of the interactions. Since this study has just begun, its success cannot yet be estimated.

These pioneering studies of Dr. Waugh and his colleagues are very exciting, since they promise to provide an approach to high-resolution structural studies of complex biological systems by means other than crystallography.

9. Local Structure of Biological Metal Complexes
GM 22793-04 (Kreilick) University of Rochester

Transition metal ions such as iron, copper, and manganese play a key role in cellular function. In all cases the transition metal must be chelated to a protein, porphyrin, or some other organic molecule to carry out its biologic function. It has become quite clear that an important key to the understanding of how these metal chelates function is the local structure surrounding the chelated metal. Since the transition metals have unpaired electrons, techniques such as electron spin resonance (esr) have been widely used to examine the interaction of chelating ligands and the metal. Although these studies are of great importance, difficulties in interpretation are considerable. Dr. Kreilick has proposed an alternative method to those currently in use. This approach is to look at ^{1}H and ^{32}P NMR spectra of powder and crystals down to 1.8°K. At this temperature, the two spin states of an electron are virtually static, and the adjacent nuclei should observe a large dipolar field. This should generate large effects in the magnetic resonance spectra of adjacent nuclei, and make it possible to identify the nucleus in question and its distance from the metal. Since the effect drops as the sixth power of the distance, a discrimination of up to 0.1Å of nuclei up to 10Å away can be obtained.

The initial award to Dr. Kreilick was for two years, to perform a feasibility study. The feasibility study has proven remarkably successful, and Dr. Kreilick is now extending his studies to more complex systems. Work already completed has demonstrated the validity of the technique using, among others, crystalline Cu(Glycine)2 H_2O and silver tetraphenylporphyhrin. In the case of copper-glycine, three well-resolved NMR signals were observed appearing at high field at temperatures below about 6°K. These indicate distances of 3.1Å, 2.88Å, and 2.7Å. The room temperature crystal structure shows an amine proton at 2.51Å from the copper, a water of hydration proton at 3.0Å, and the methylene proton at 2.5Å. This is reasonable agreement considering the differences in experimental conditions, and the preliminary nature of the calculations. For the silver porphyrin, the distance from silver to the pyrole protons was calculated as 5.2Å from the NMR, compared to 5.1Å from X-ray.

More recent work has provided much additional supporting data. This includes includes the observation of distinct differences between copper-alanine, copper-valine, and copper-glycine, indicating that such studies of metalloproteins will unambiguously show the type of amino acid coordinated to a metal atom. In short, this method promises to be of great importance to those interested in metalloprotein structure and function.

10. Bose - Einstein Condensation in Biological Systems
GM 24502-01 (Wu) State University of New York at Albany

Dr. Tsu-Ming Wu, of the State University of New York at Albany, has a grant in this program area for his work on "Bose-Einstein Condensation in Biological Systems." This work, still in its first year, appears to be very specialized and esoteric. It is, however, directly relevant to a serious national health question. Dr. Wu's work should provide the basis for an experimental examination of a theory that attempts to explain possible biological efects of low microwave radiation.

Biological systems such as membranes, proteins, and nucleic acids contain electric dipoles that are capable of electric oscillations. Long range Coulomb interactions between these large dipoles can establish electric vibration modes. The theoretical physicist, Frolich, has suggested that if energy is supplied to the system under certain conditions, large amounts of energy might be channeled into the lowest energy mode. This physical phenomenon is called Bose-Einstein condensation and contrasts to the usual distribution of vibrational modes throughout the broad range of oscillation frequencies. A strong coherent electromagnetic oscillation would be established, possibly disrupting membranes and other molecular structures. This theory is one of few suggested mechanisms capable of explaining the potential damage to biological systems by low microwave radiation. Although the Frohlich theory is considered speculative, it has received support from the recent Russian publication of microwave data showing strong frequency dependent kill rates on various tissue cultures and increases in cell division.

Dr. Wu has developed a correct quantum mechanical description of the Frohlich theory, which was originally developed from rate equations and, in Dr. Wu's view, contains ambiguities. Dr. Wu now proposes to examine the implications of his formulation and to construct models for biologicl structures that will permit meaningful experimental comparisons.

11. Crystallographic Analysis of Protein Structure -
GM 15000-10 (Richardson), Duke University

David and Jane Richardson, are well known protein crystallographers at Duke University. Their research has been focused on the determination of the three dimensional structure of superoxide dismutase. This class of widely distributed enzymes provides protection for aerobic organisms and tissues against free radical damage from the superoxide radical (O_2^-). This occurs by the conversion of the radical to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). There are two major classes of this enzyme known. The first is Cu, Zn superoxide dismutase, which is found in the cytoplasm of a wide variety of eukaryotes. This protein has two identical subunits, each having a molecular weight of 16,000 and with both a Cu atom and a Zn atom at the active site. The second class is Mn, Fe superoxide dismutase, which is found in prokaryotes and the mitochondria of eukaryotes. Each of these protein molecules consists of four subunits of molecular weight 20,000 with either Mn or Fe at the active site.

Applying the standard x-ray crystallographic techniques that have been developed for large protein molecules, the Richardsons have calculated an electron density map at medium resolution for bovine Cu, Zn superoxide dismutase. Such a map does not permit the resolution of individual atoms, but does enable experienced protein crystallographers like the Richardsons to trace the polypeptide chain, locate the side chain of each amino acid, and determine the overall structure of the protein. The dominant feature of this protein is a cylindrical barrel formed by a large -pleated sheet. The latter is a periodic structure common to many proteins and is composed of segments of the polypeptide chain. The interior of this barrel is packed with hydrophobic side chains. In each subunit the Cu atom and Zu atom are found close together at the active site. The Zn atom is bound in a tetrahedral configuration to the imidazole rings of three histidines and to one aspartic acid residue. The Cu atom is bound to one of the above histidines plus three others in a square arrangement. The Richardsons have collected high resolution data and are utilizing the University of North Carolina computer graphics facility (supported by the Division of Research Resources) as well as computer refinement techniques to determine accurately the atomic coordinates of bovine Cu, Zn superoxide dismutase. Hopefully, with an accurate model of the structure, stereochemical considerations will reveal the mechanism of enzymatic activity. In addition to the above work, the Richardsons have begun work on an example of the second class of this enzyme, namely human liver Mn superoxide dismutase. A comparison of the two structures will be interesting, since the two enzymes are almost identical from a biochemical point of view, but have quite different physical properties.

During their analysis of bovine Cu, Zn superoxide dismutase, the Richardsons have discovered overall structural relationships in the folding of different proteins and several general structural features common to numerous proteins. In the first category, folding similarities have been found between: (1) Cu, Zn superoxide dismutase and immunoglobulins, (2) soybean trypsin inhibitor and papain, (3) snake neurotoxins and wheat germ agglutinin, and (4) globins and thermolysin. These close structural relationships, even with no sequence homology, might imply an evolutionary connection. On the other hand, similar proteins might be produced independently if certain topologies happen to be superior for the construction of a protein, and thus would be found in many very different proteins. The Richardsons' work lends some credence to this latter case. They have analyzed β-pleated sheets in all known protein structures. Adjacent chains of β sheets can run either in the same direction (parallel) or in opposite directions (antiparallel). The Richardsons' survey has shown that mixed cases rarely exist, and that parallel sheets are always found only buried inside large proteins. Another example of their findings involves connections between chains of parallel β sheets. Each connection has to cross over the sheet and can be classified either as right or left-handed. Although there is no apparent advantage of either case, the Richardsons have found that 97% of the crossovers are right-handed. They have found several other common features of β sheets and are now generalizing their work to produce a classification scheme that places all known protein structures into four major categories. This work is facilitated by schematic drawings they have made of all the 80 or so known protein structures, several

of which are shown in the Figure. In the case of lactate dehydrogenase, the arrows represent a six segment parallel β-pleated sheet found in the protein's interior and the coils represent one short and four long α-helices at the proteins exterior.

This research project has not only led to the structural solution of an important enzyme, but has also produced a scheme for comparing all known protein structures.

12. Microtubule Associated Proteins (MAPs)
GM 14642-13, Joel L. Rosenbaum, Yale University

Tubulin is the major protein subunit of microtubules, which exist in essentially all cells. Microtubule-associated proteins, or "MAPs", are those proteins which co-purify with tubulin during successive cycles of assembly and disassembly of microtubules in vitro. This nomenclature is used principally in respect to the high molecular weight proteins which co-purify with in vitro-assembled brain tubulin, but it is also used to describe any protein which maintains the same stoichiometry to tubulin during cycles of assembly and disassembly, e.g., the "Tau" proteins which are of lower molecular weight also co-purify with tubulin isolated by assembly from tissue culture lines such as HeLa.

The MAPs, at least the high molecular weight MAPs purified from brain cells, are considered highly significant in that it has been shown quite clearly that they form the periodic, arm-like structures associated with the walls of microtubules. This is readily photographed by electron microscopy. That these arm-like structures are not artifacts of the in vitro assembly procedure has been demonstrated by making antibodies to them, labeling the antibodies with fluorescein, and then showing that the tagged antibodies will label microtubules in situ. These MAP antibodies label the microtubules of the mitotic apparatus, as well as the microtubules of the cytoskeleton of various tissue culture cells.

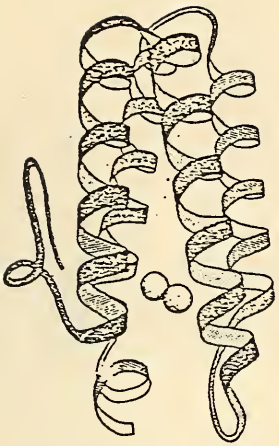
The functional significance of the MAP-containing arms on the microtubules is not known, but three possibilities have been advanced on the basis of in situ location of microtubules: (1) They may be involved in the bridging of microtubules to each other or to other structures in the cell, such as intermediate (100 Å) filaments, or the cell membrane - strictly for skeletal or support purposes; (2) The MAPs may be analogous to the Dynein-ATPase-containing arms associated with the ciliary and flagellar microtubules, which provide the motive force for the sliding of the flagellar microtubules against each other; (3) The MAPs may provide attachment sites on the microtubules for actomyosin to provide the motive force for microtubule-based movements. There is a certain amount of ultrastructural and biochemical evidence in support of all of these possibilities.

Among other studies, Dr. Joel L. Rosenbaum and his students at Yale have been investigating the possibility that the function of the MAPs is analogous to the arms on the flagellar microtubules called dyneinATPase, which are responsible for providing the motive force for microtubule sliding and thereby flagellar movement. In his laboratory, they have demonstrated that

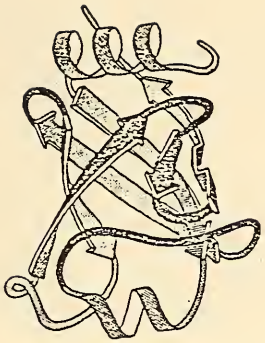
one can remove the flagellar dynein arms from Chlamydomonas or sea urchin flagella, and then add the MAPs from brain microtubules to the flagellar microtubules and show that the flagellar microtubules are no longer capable of binding the dynein. They have also been able to show that the MAPs from brain microtubules bind to brain microtubule wall with a periodicity which is almost identical to the periodicity of binding of dynein to the flagellar microtubule wall. More recently they have shown quite clearly that the flagellar dynein can bind to the walls of the brain microtubules which have been stripped of their MAPs, and that the binding occurs not only with the same periodicity as on flagella, but the angle at which the dynein binds to the cytoplasmic microtubule wall is similar to its binding angle on the flagellar microtubule wall. The fact that the dynein binds to the surface of the cytoplasmic microtubules periodically and at an angle, gives the microtubule an "arrow-head" appearance, much like the decoration of actin with heavy meromyosin. Because of this, it is now possible to determine the polarity of microtubules in any situation simply by binding dynein to them and observing the directionality of the arrowheads (PNAS, in press). Moreover, when one binds the dynein to cytoplasmic microtubules in vitro, the tubules become cross-linked to one another by the dynein arms. The cross-linked microtubules can then be dissociated by adding ATP -- much in the same way that dynein-bridged flagellar microtubules can be dissociated by ATP. Thus, dynein attached to cytoplasmic microtubules shows many of the same characteristics that it does when bound to flagellar microtubules. This does not mean, however, that dynein is present in situ on these cytoplasmic microtubules. One of the ways to test this will be by making fluorescent antibodies to dynein and reacting them with various cytoplasmic microtubule systems. Indeed, workers in Japanese laboratories have already done some of these studies and have shown the presence of dynein in the mitotic spindle. More of these studies are required, using cells such as the neuron and melanosomes.

Perhaps one of the more provocative reports suggesting that the MAPs may be analogous to flagellar dynein-ATPase is recent work showing that one of the high molecular weight of in vitro-assembled brain microtubules may be associated with an ATPase (this is not to say that the MAP is the ATPase, only that it is associated with the activity). This work is just in its initial stages, but the MAP-ATPase activity appears to have much in common, in terms of enzymology, with the flagellar dynein ATPase, according to Dr. Rosenbaum.

In summary, then, there are three possible functions for the MAPs: (1) structural, (2) as a foundation for the attachment of actin/myosin, or (3) as dynein-like arms. All three possibilities are the objects of current research in which a variety of microtubule-based translocation systems are being used: the mitotic apparatus, nerve axons, and the pigment particles of epidermal cells of amphibians and fishes. The approaches are multiple: fluorescent antibodies, in vitro assembly and reconstruction of the motile system in the test tube, reactivation of intact systems to obtain movement upon the addition of ATP, classical biochemical analysis of the components of the system, and combinations of these approaches. The field has been moving very quickly and it is expected that in the reasonable future we will have some clear ideas concerning the motive force for microtubule-based movement.

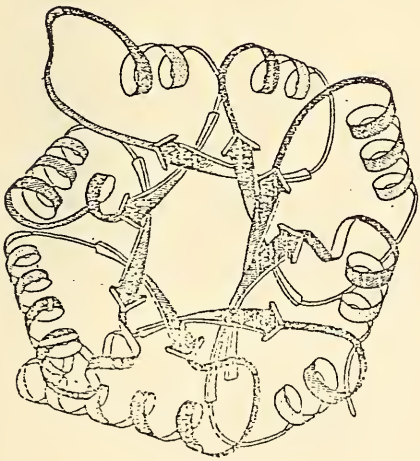


Myohemerythrin

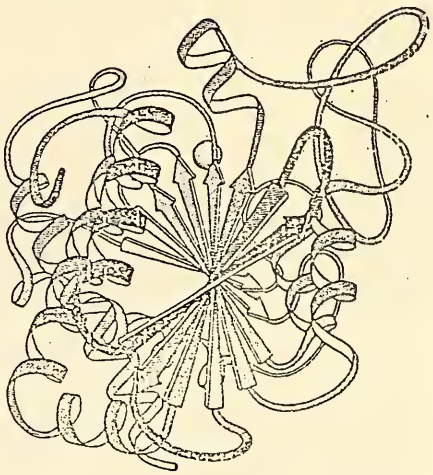


Papain, domain 2

Lactate Dehydrogenase domain 1.



Triose-P Isomerase



Carboxypeptidase

Crystallographic Analysis of Protein Structure
(see Highlight # 11)

13. Cell Division and Membrane Proteins
GM 19043-08 (Masayori Inouye) SUNY, Stony Brook

Dr. Masayori Inouye has isolated and purified a membrane lipoprotein and its precursor from E. coli. This was achieved by purifying the complete mRNA for the prolipoprotein (precursor) and directing synthesis in a cell-free wheat germ system. The resulting lipoprotein was completely sequenced, paracrystallized and it's ultrastructure studied. Because of the knowledge of its sequence, and the ability to control its synthesis in some coli strains, it has been possible to incorporate in the protein both spin and NMR label selectively in vitro. A close association between the lipoprotein and the other major E. coli membrane protein, matrix protein, has been demonstrated. Additionally, the NMR labeled side chains have been shown to be free moving. Dr. Inouye has mapped the gene at 36.5 on the coli linkage map. By sequencing the mRNA from both ends, he has disclosed large nontranslated regions at both 5' and 3' ends. This section of the 5' end contains a sequence identical to that which contains the 80s ribosome binding site in eukaryotic systems.

From the amino acid sequences of the peptide extensions of prolipoprotein and three other protein precursors, a set of four common rules were deduced. The precursor leaders have: a) a basic amino terminal section; b) a stretch of hydrophobic amino acids following this basic section; c) a proline or glycine residue within this hydrophobic section; and d) an amino acid with a short side chain at the cleavage site. These homologies suggest that the peptide extensions are required for translocation of the secreted protein across the cytoplasmic membrane and possibly not for determination of final location in the envelope. Dr. Inouye has developed a 'loop model' for protein translocation.

Through the efforts of Dr. Inouye's laboratory, we may know more about some E. coli proteins than any other membrane proteins from other cells. For this lipoprotein we know something of the genetic locus, mRNA sequence, in vitro synthesis, conditions of in vivo synthesis and control, amino acid sequence, mechanism of membrane insertion, mobility in the membrane, and interaction with other membrane proteins and lipids. He also has several mutants. Some plasmids of membrane proteins have been cloned and even inserted in phages. Dr. Inouye's ultimate goal is to understand the mechanism of bacterial cell division. With his record of productivity and detailed, multilevelled analysis, he may well succeed.

INSTRUMENTATION

The past three Annual Reports for the CMBD Program included initial discussions on the rapidly growing dependence of investigators on high technology, high-cost instruments essential for the conduction of research in cellular and molecular biology. Progress was reported on a workshop organized by the Program which summarized the concerns and need for updating existing and providing new instruments for this increasingly quantitative research. We can report this year that, resulting from these earlier efforts, the Institute announced and funded a new NIGMS Shared Instrumentation Program in the amount of $7.4 million.

Sixty five awards were made to research groups at 55 institutions providing for 12 electron microscopes, 12 nuclear magnetic resonance instruments, 10 circular dichroism machines, 7 mass spectrometers, 5 X-ray and/or computer graphic systems, 8 protein sequencers and/or amino acid analyzers, 5 cell sorters, and 6 other awards for instruments serving fluorescence microscopy, electron paramagnetic resonance, ultrasonics and scintigraphic radiological evaluation. These shared instruments will significantly enhance the research capabilities at the cellular and molecular level of hundreds of investigators currently supported by the NIGMS as well as grantees of other NIH Institutes.

CONFERENCES - FY '79

In FY '79, the National Institute of General Medical Sciences, through the CMBD Program, funded partial support for four conferences. These meetings are of direct relevance to the goals of the CMBD Program and topics included transmethylation, membrane biogenesis, structure of lipoproteins, and iron and proteins in O_2 and electron transport.

RESEARCH TRAINING

The Cellular and Molecular Basis of Disease Program supports predoctoral and postdoctoral research training in the broad range of fields encompassing cellular and molecular biology. Approximately one-half of the total NIGMS training effort is administered by the CMBD Program. In turn, the Institute accounts for two-thirds of the predoctoral trainees supported by all of the NIH. Research training at NIGMS, as well as at the whole of NIH, is supported under the authority of the National Research Service Award Act of 1974. With the advent of that legislation, predoctoral research training at the NIGMS has been in the form of interdisciplinary or interdepartmental training grants, while postdoctoral support is provided both through training grants and individual fellowships. The research training program is essential for the long term mission of the CMBD Program since it fosters the development of a cadre of first class young scientists in the areas of cellular and molecular biology. These scientists will be the research leaders over the next several decades.

Predoctoral training in the CMBD Program is provided in two areas: the Cellular and Molecular Biology Training Program and the Medical Scientist Training Program. Training support for postdoctorals is provided through institutional training grants in Basic Pathobiology and through individual fellowships by the Cellular and Molecular Biology Fellowship Program.

1. Cellular and Molecular Biology Training Grants - 55 grants - 1,268 trainee positions, $ 11,401,500

   These multidisciplinary training grants are designed to provide broad, fundamental research training in the biomedical sciences. For most grants, the training faculty are selected from many departments on the basis of their excellence in research and research training. The Cellular and Molecular Biology training grants are unique in their broad coverage of the biomedical sciences, with faculty and students from many fields, such as biochemistry,

biophysics, microbiology, immunology, biology, bacteriology, cell biology, molecular biology, physiology, pharmacology, medicine, genetics, anatomy, pathology, and the neurosciences. A majority of these training grant programs are beginning their final year of support and have submitted renewal applications. The Cellular and Molecular Basis of Disease Committee is currently reviewing these applications, with overriding emphasis on quality -- of the faculty, the trainees, and the program being conducted.

2. Medical Scientist Training Grants - 23 grants, 616 trainee positions, $7,568,500

   The Medical Scientist Training Program is unique and has been singled out and lauded by the National Academy of Sciences/National Research Council's Committee on A Study of National Needs for Biomedical and Behavioral Research Personnel. This is a special, separate category of predoctoral training grants which are awarded to medical schools and affiliated graduate schools for support of candidates for the M.D./Ph.D. degree. These trainees have access to thesis research opportunities in the biomedical and health-related sciences represented at the respective grantee institution.

3. Basic Pathobiology Program - 20 grants, 130 trainee positions, $ 2,204,000

   This program supports 20 training grants, and for the past year 130 trainee positions were provided. Analysis of this program shows only 17% of these trainees are M.D.'s. The program's intent was that M.D.'s constitute 50% or more of the trainees. Attention has been given to reorient the program in this direction during the coming year.

4. Cellular and Molecular Biology Individual Fellowships - 91 grants, 91 trainee positions, $ 1,232,500

   The postdoctoral National Research Service Awards in the Cellular and Molecular Biology Fellowship programs are provided to enable individuals holding the Ph.D. to acquire special advanced research training toward developing necessary cross-field knowledge for a research career in cell sciences. We continue to see high qualtity applications that encompass the entire spectrum of the program in areas such as membrane structure and function, cell motility, differentiation and growth, enzyme catalysis and regulation, and proteins and other macromolecules which are essential for an understand ing of living systems at the cellular-molecular level.

GENETICS PROGRAM

Objectives

The objectives of the Genetics Program are to support research on the structure, synthesis, expression and control of the hereditary material of the cell at all levels of organization from the molecular and submolecular to the cellular and organismic. The ultimate purpose of such research is to develop a better understanding of genetic mechanisms and the role of heredity in normal and abnormal life processes. Many diseases have been shown to result from the altered activity of one or more genes and it is expected that a greater understanding of how genes act and how their activity is controlled will contribute to the prevention and control of such diseases.

Program Activities

Scientifically, the year has been an exciting and productive one for the research area supported by the Genetics Program. Recombinant DNA technology continues to have a dramatic impact on the rate at which knowledge is accumulating, although some areas are blossoming even without the aid of these new techniques. The highlights section describes a sampling of interesting and significant results in areas that have been particularly active.

In the fall of 1978, the Program sponsored a workshop to discuss the state of research on the role of genetics in common diseases. The workshop was attended by ten consultants and representatives from several other NIH Institutes, as well as by staff of NIGMS. The major conclusion of the discussions was that approaches to the study of complex diseases were being developed and that the role of genetics in such diseases was becoming clearer. In fact, the degree of enthusiasm about the opportunities in this area of research was such that the consultants recommended several mechanisms to NIGMS for stimulating progress in the field. These mechanisms included support of conferences and workshops, training of manpower, and announcement of NIH interest in research applications for studies on the genetics of common diseases. The Program intends to pursue these suggestions and implement those that hold most promise.

Since the workshop our optimism for this area of research has, if anything, increased. We have funded a grant to the University of Utah for the development of a computerized data base founded on the Mormon genealogical records. This resource is intended to be used for genetic and epidemiologic studies of common diseases by the most advanced methods available and promises to be very useful in this regard. One of the key needs of this field is a more comprehensive set of easily measured human polymorphic markers which can be utilized in linkage studies to trace the inheritance of susceptibility to particular diseases in families or populations. Methods for discovering such markers through restriction enzymes and recombinant DNA technology are now being developed and will undoubtedly produce many exciting findings for future annual reports. This development will bring human population genetics and epidemiology into the molecular era and may have a profound impact on research in human genetics.

As a result of the flexibility of this Institute in allowing the scientific interests of program administrators to shape program areas, we have been able to bring into close coordination a portion of the Genetics Program, the genetic aspects of cell organelles, with a portion of the CMBD program, bioenergetics. This is accomplished by appointment of Dr. David Beck to the staff of both programs. The production of energy by oxidative or photophosphorylation is the prime function of the commonly studied organelles, the mitochondria and the chloroplast. The central nature of this process in biology and its yet unresolved mechanisms make it a prime focus of biochemical study. In addition, study of the genetic apparatus in these organelles and its relation to the nucleus is essential for an understanding of the control of genes specifying the energy productive systems. Cell organelles are also proving useful in providing a general understanding of the interaction of nuclear and cytoplasmic genetic systems in eukaryotes.

Research applications received in the organelle genetics program often overlap extensively with the research goals of the bioenergentics program. The advantage of having the two programs under one administrator is that a clearer overview of both the successes as well as the potential of these research fields is possible. In the past, research in bioenergetics has centered on the biochemical mechanisms of energy conservation while the genetics has been concerned with sites of coding for and control of assembly of the overall energy conservation apparatus.

With elucidation of the role of transport enzymes and the integration of theories provided by the Mitchell hypothesis, both biochemists and geneticists in the area have renewed their efforts to understand the role of the genetic specification of organelle proteins in ion transport, assembly of a functional electron support chain, and developmental alterations in cellular bioenergetic properties.

In the research training area, the Program sponsored a meeting of the Directors of postdoctoral training programs in genetics (with emphasis on medical genetics) in the spring of 1979. The purpose of this meeting was to discuss the problems and opportunities of training for medical genetics research and to exchange views about this subject between Program Directors and Institute staff. The Program Directors described the characteristics of their particular training grants and the kinds of individuals they are training. It was clear that, while the training grants varied a great deal one from the other, the quality of training was generally very high. The commonly perceived problem of attracting a sufficient number of individuals with medical training (M.D.'s) into research on medical genetics was discussed at length and everyone agreed more effort should be made to attract such individuals. The Program Directors felt they had benefited from the exchange of ideas with each other and staff welcomed the opportunity to hear firsthand reports about the state of the field.

As in past years, we have experienced some turnover in professional staff during FY 79. Dr. Jerry Roberts departed for a position with the Special Study Section program in DRG. In his stead, Dr. Robert Gulley joined the Program in June 1979. Dr. Fred Bergmann, Program Director, was absent on a detail to the Office of Technology Assessment, United States Congress, from May 1979 through the end of the fiscal year. During his absence Dr. Elke Jordan, Deputy Program Director, was the Acting Director.

RESEARCH HIGHLIGHTS

A. The recA Protein: Importance in DNA Repair, Genetic Recombination, Mutagenesis and Viral Gene Expression
GM 09901 (Philip Hanawalt) Stanford University
GM 06196 (Robert Lehman) Stanford University
GM 24178 (John Little) University of Arizona
GM 24496 (David Mount) University of Arizona
GM 21941 (Jeffrey Roberts) Cornell University

There are two main types of genetic recombination in bacteria. One is generalized recombination, that is recombination that can take place, as far as is known, at any site of the chromosome. The other is site specific or specialized recombination which takes place only at particular sites and is involved in such processes as virus integration into the chromosome, movement of transposons from one location to another, etc. (See Section B). The two systems are genetically quite distinct.

Generalized recombination is affected by many genes but the major gene controlling the process is known as recA. This gene and its product, the recA protein, have been the subject of major discoveries in the past year. Although a great deal is known about the genetics of recombination, very little is known about its biochemistry. It has been clear for some time, however, that genetic recombination in both bacteria and eukaryotes involves a reciprocal exchange of pieces of DNA between homologous chromosomes. It is remarkable that the cell can carry out this reaction without gaining or losing any genetic information and without introducing any errors. In addition to recombination, the recA gene controls a number of other cellular functions and links them into a biochemical network. These other reactions include sensitivity to UV, prophage induction, mutation, DNA repair, DNA breakdown. Many of these reactions are observed only after damage to the host DNA and are induced by it, as is recA itself. Because of this, these reactions have been nicknamed "SOS functions."

RecA mutants were discovered about 15 years ago by Clark and Margulies. Although originally selected on the basis of decreased recombination efficiency, the mutants were subsequently found also to be deficient in a number of other important cellular processes. They were first found to be extremely sensitive to ultraviolet light, ionizing radiation, and chemicals that damage DNA. Thus, the recA function was required not only for genetic recombination, but

for DNA repair as well. This requirement can now be explained by the observations of Dean Rupp and his colleagues who showed that damaged chromosomes were only partially replicated because the DNA synthesizing apparatus of the cell appeared to skip past damaged sites on DNA, leaving gaps. Gaps were subsequently filled in, at least in part, by genetic recombination between one partially replicated chromosome and the sister chromosome copied from the other parental DNA strand. This process had the effect of removing replication gaps and diluting the damaged sites each round of chromosome replication so that a further round of replication was possible, eventually giving rise to damage-free progeny chromosomes.

In addition to their deficiencies in genetic recombination and DNA repair, recA mutants were shown to have three other abnormalities. First, they were defective in induction of proviruses, such as bacteriophage lambda. In the normal E. coli cell harboring a provirus, treatments which damage DNA or inhibit DNA synthesis lead to lytic virus development. A virus-coded repressor, which shuts off the lytic genes in the uninduced cell, is destroyed, thereby allowing transcription of the lytic viral genes by the host RNA polymerase. In cells carrying a recA mutation, destruction of the virus repressor does not occur after inducing treatment.

Another defect found in recA-mutants is in a process called inducible DNA repair. If wild-type virus-free E. coli cells are given the same kinds of treatments which induce a provirus, they have an increased capability to repair bacteriophage which have been treated with a DNA damaging agent before infection of the pretreated cells. This new capacity for DNA repair requires protein synthesis as well as the recA function.

Finally, it was found that a variety of treatments which are mutagenic for normal E. coli cells are not mutagenic in recA cells, e.g., irradiation by ultraviolet light. A metabolic pathway which makes errors in repairing damaged DNA exists in wild-type cells, but is defective in recA mutants. Radman and his colleagues have proposed that DNA polymerase is modified in the mutagenized cell so that it is better able to replicate past damaged sites on the DNA. Such a mechanism is advantageous to the cell in that it allows replication past damaged DNA, but has the disadvantage that incorrect DNA bases are inserted opposite damaged sites, giving rise to mutations after further chromosomal replication.

The extreme pleiotropy of recA mutants suggests an important biochemical role for the protein and has led a number of investigators to search for the recA product so that its biochemical activity can be analyzed. The protein was discovered three years ago by McEntee using recA transducing bacteriophage and was shown to be a polypeptide of approximately 40,000 daltons molecular weight. Shortly after this discovery, this polypeptide was found to be the same one as a major polypeptide synthesized at a very high rate in cells given the same kind of inducing treatments as described above. This polypeptide, designated Protein X, had been discovered five years earlier by Inouye and Pardee, and much information had been accumulated as to how its synthesis was regulated. A variety of genetic and biochemical studies have suggested that synthesis of the recA polypeptide is repressed in uninduced cells by the

product of another DNA repair gene, lexA. In induced cells, the lexA repressor appears to be destroyed in a fashion similar to that by which the phage repressor is destroyed. Two kinds of lexA mutants support this regulatory model. One class is extremely UV-sensitive and deficient in mutagenesis and recA protein induction. The other class is constitutive for recA protein synthesis. These lexA mutations are thought to be analogous to mutations which have been described in other repressor genes in E. coli.

Recently, Ginsburg and Mount have isolated mutations at the recA locus which make recA protein synthesis refractory to the influence of the lexA gene product. These could define an operator region to which lexA repressor binds. The recA regulatory region has been mapped extensively by restriction enzymes in several laboratories and the nucleotide sequence determined by Rupp and Ogawa. Mutants with lowered promotor efficiency have also been described. Consequently, it should now be possible to sequence a variety of mutants in order to arrive at a structure-function map of the recA regulatory region.

Additional biochemical studies by Little and Mount and their colleagues have led to the cloning of the lexA gene and the identification of a mutant product as a 25,000 dalton polypeptide. Attempts to demonstrate that this protein is a repressor of recA have been thwarted by the apparent instability of the protein and by the fact that it is made in very small amounts by the cell.

Several laboratories have been analyzing the biochemical properties of purified recA protein. Roberts has shown that recA protein is a protease which cleaves phage lambda repressor in vitro in an ATP-dependent reaction. The cleaved repressor will no longer bind to DNA. Thus, in the E. coli cell inducing treatment leads to the synthesis of a large amount of recA protein which destroys the viral repressor and turns on the lytic viral functions. The reaction between recA protein and lambda repressor in vitro requires single-stranded DNA, and Roberts has recently shown that the molar ratio of recA protein to DNA nucleotides is a key factor in determining the rate of proteolytic activity of recA protein. Hence, the intracellular level of recA must be responsive to the extent of DNA single strandedness, if repressor cleavage is to be efficient.

At the 1978 Cold Spring Harbor meeting, Roberts and Ogawa independently described an ATP-ase activity of recA protein. This activity has proven useful for extensive purification of the protein. The laboratories of Lehman and Radding have shown that purified recA protein catalyzes the pairing of homologous chromosomes. The assay measures the insertion of single strands of DNA into homologous supercoiled or nicked circular DNA's. The product can be detected by its binding to a nitrocellulose filter in the presence of high salt concentrations. This in vitro reaction has requirements similar to the protease of Roberts. ATP is required and the molar ratio of recA monomer to DNA nucleotides must be similar. The apparent role of recA protein in this reaction is two-fold: first, it appears to be able to open and/or stabilize denatured regions in double-stranded DNA, and secondly, it appears to remove secondary structure in single-stranded DNA, allowing it to penetrate the denatured homologous region in a double-stranded DNA molecule.

These biochemical properties of the recA protein can account for many of the deficiencies of recA mutants. Still not understood is the role of recA protein in inducible DNA repair and mutagenesis. Conceivably, it could act as it does in recombination, stabilizing DNA structure so that damaged DNA can be more efficiently replicated. Alternatively, it could act as a protease in, modifying proteins necessary for replication, thereby altering replication fidelity. However, no cellular protein which is susceptible to the protease has yet been found, although such susceptible proteins can presumably exist.

Studies of recA protein function in DNA repair and mutagenesis have been spurred on by the discovery in the laboratories of Summers, Hanawalt and others of an inducible DNA repair process in mammalian cells. Monkey cells lightly stimulated by irradiation with ultraviolet light or carcinogenic chemicals repair damaged SV40 or herpesvirus much more efficiently than untreated cells.

B. Transposable Genetic Elements
GM 26527 (Robert Rownd) University of Wisconsin
GM 23614 (Gerald Rubin) Sidney Farber Cancer Institute
GM 24960 (James Shapiro) University of Chicago

Until a very few years ago, most biologists believed that the order of genes on chromosomes was fixed in all the cells of an organism. While chromosome rearrangements were known to occur and to affect the properties of an organism, they were called "aberrations" and thought to be rare, "freak" events in the history of a species. Today we realize that chromosome rearrangements occur in many organisms much more often than previously realized. Considering mammalian biology, we know that some rearrangements are part of normal developmental processes (such as the expression of the immune system) while other rearrangements are detrimental and associated with specific pathological states (such as leukemias). Chromosomal rearrangements have also played an important role in evolution.

One of the most revolutionary discoveries in molecular genetics in the past decade is that a number of specific DNA elements existing at multiple locations on prokaryotic genomes can transpose or translocate spontaneously to new sites by a mechanism that is independent of the normal recombination system (recA) of the host. These specific elements or "insertion sequences" (IS) range in size from 700-1400 bp and several have already been sequenced. They may contain DNA sequences in inverted repetition near their ends which may have functional significance because of their ability to insert in either orientation at many different sites on chromosomes. When they insert into a structural gene of a bacterial operon, IS elements may inactivate not only that gene but also other structural genes of the operon which are distal with respect to the regulatory genes of the operon. The formation of such polar mutations was how IS elements were originally recognized.

Transposons (Tn) are larger segments of DNA, which in addition to their ability to move from one genetic site to another, including from one chromosome to another, also contain genes for resistance to antibiotics. These transposable elements are thought to be responsible for the epidemic spread of drug resistance in bacteria. Certain bacterial viruses, including phages lambda and Mu, can insert into the bacterial chromosome at different sites

and are therefore viewed as transposable elements. Similar transposable elements have also been demonstrated in higher organisms, but these are much more difficult to analyze. These elements include "controlling elements" in maize, Drosophila transposons, and certain tumor viruses.

Transposons were originally found in the drug resistance plasmids, but these elements are now also known to exist in other bacterial plasmids, bacterial viruses, and even the E. coli chromosome. The resistance genes in these elements are usually flanked by inverted or directly repeated sequences, some of which have been shown to be identical to insertion elements. Both IS elements and transposons have the ability to move into DNA sequences as intact units, indicating that the ends of these elements act as sites for recombination. In fact, small bacterial chromosomes called R factors have been shown to act as hot spots in the formation of deletions of large segments of host DNA adjacent to the translocatable DNA.

1. The Mechanism of Transposition
GM 24960 (James Shapiro) University of Chicago

Shapiro has been studying the mechanisms by which transposons enter and leave the bacterial chromosome. He is studying two drug resistance transposons, Tn5 which encodes for kanamycin resistance and Tn9 which encodes chloramphemicol resistance, and the IS1 element. The last two elements are related because Tn9 consists of two IS1 elements bracketing a chloramphenicol acetyl transferase gene.

IS1 was first implicated in mediating transposition events by Shapiro's observation that Tn9 could replace the chromosomal integration system for phage lambda. The IS1 elements were shown to serve as sites for insertion of phage lambda into the chromosome and also for its excision. During the integration of phage lambda, at least one of the IS1 elements is duplicated in the process. Exit of the phage is efficiently mediated by reciprocal recombination of the flanking elements.

Shapiro has postulated a mechanism for transposition wherein the insertion element is cleaved at its outer extremity and the target DNA molecule is cleaved at sites chosen to yield 5- or 9-bp cohesive ends. Ligation takes place in such a way as to yield an X-shaped structure held together by the duplex of the transposable element. Next, there is filling in of the impaired oligonucleotide sequences at the ends and semi-conservative replication of the transposable elements. Polymerization and completion of this replication requires no new biochemical activities, but specific proteins may be involved. Finally, site-specific reciprocal recombination takes place between the (now) two transposable elements to generate both the original donor molecule (with some newly synthesized material in the transposon) and the target molecule containing an inserted element flanked by a 5- or 9-bp repeat. Although homologous recombination systems can catalyze this step, there must be some recA-independent process because Shapiro has found that transposition can occur in recA- strains.

Shapiro's hypothesis is still being tested, but it is consistent with most known events occurring during transposition. Furthermore, the model

allows for replication only of the transposable elements, an important conceptual breakthrough to understanding spread of transposable elements (and their drug resistance factors) which occurs in the absence of replication of the entire genome. This point is also significant in that this selective replication parallels a developmental chromosome rearrangement system that controls sexuality in the eukaryote, yeast.

2. DNA Insertion Sequences and Gene Amplification
GM 26527 (Robert Rownd) University of Wisconsin

The role of IS elements in the formation of genetic rearrangements within bacterial drug resistance plasmids is being investigated in the laboratory of Robert H. Rownd at the University of Wisconsin. The drug resistance genes of some R plasmids are clustered within a segment called r-determinants which is flanked by copies of IS elements all oriented in the same direction.

After culture of host bacteria in medium containing appropriate drugs, the R plasmid DNA is found to contain multiple, tandem copies of r-determinants which are joined to a single copy of the resistance transfer factor (RTF) segment of the plasmid. The RTF harbors the replication and transfer genes. This selective amplification of r-determinants results in a large increase in the drug resistance of the host cells, owing to gene dosage effects. The IS elements which flank r-determinants appear to serve as recombination sites during at least the initial stages of drug resistance gene amplification. Rownd's results with r-determinants are consistent with the model for movement of transposable elements proposed by J. Shapiro (See Section B-1 above.

Once tandem copies of r-determinants are formed, homologous recombination between different r-determinant units may mediate the plasmid rearrangements resulting in further drug resistance gene amplification. Recombination involving IS elements is recA-independent, whereas recombination involving homologous regions of r-determinants appears to be recA-dependent. IS elements have also been found to serve as "hot spots" for the formation of various types of plasmid deletions by a recA-independent mechanism. One R plasmid which has been studied appears to contain as many as nine recombination sites which mediate the amplification of different combinations of durg resistance genes. Most of these sites are located in close proximity to known IS elements or repetitious DNA sequences on the plasmid. These findings suggest that different IS-like elements may interact to mediate the selective amplification or deletion of segments of plasmid DNA.

A number of plasmids in different bacterial genera have been found to undergo drug resistance gene amplification, and repetitious DNA sequences which flank drug resistance genes appear to serve as recombination sites. Tandem duplications of segments of bacterial chromosomes have also been observed. Amplification of dihydrofolate reductase gene and increased resistance to the folate analog, methotrexate, has also been reported for mouse cells which have been cultured in the presence of the analog (See Schimke, Section E-3 below). The reversible amplification of specific segments of chromosomes may be of widespread occurrence in both procaryotic and eucaryotic cells. These genetic rearrangements could be involved in cell differentiation and differential gene expression.

3. Transposons in Drosophila
GM 23614 (Gerald Rubin) Sidney Farber Cancer Institute

The existence of transposable genetic elements in eukaryotes has been suggested as a means of explaining certain genetic phenomena in maize, Drosophila, and yeast. Until recently, however, biochemical evidences in support of such elements in the eukaryotic genome has been lacking. During the past year, Gerald Rubin, has demonstrated that elements of two different dispersed repeated gene families are transposable elements in the Drosophila melanogaster genome. These families, termed 412 and copia, code for abundant poly A-containing cytoplasmic RNAs.

Each of these dispersed repeated gene families consists of thirty to fifty copies of one DNA sequence, the gene element, repeated faithfully at thirty to fifty distinct and widely separated chromosomal loci. It is in the dispersed character of these gene families that they differ from other examples of multiple copies which cohabit a single genome, such as the ribosomal RNA and histone genes. In the latter two examples, the genes are tandemly repeated DNA units, and each copy is contiguous with the others. The most striking feature of elements of the dispersed repeated gene families, however, is that their number and chromosomal locations are not constant; their positions differ widely both between the genomes of embryos and tissue culture cells, and between the different strains of Drosophila melanogaster. This apparent plasticity of the genome is further indicated by the variance in number and location of these elements between individuals of the same strain. Using recombinant DNA techniques, Dr. Rubin has cloned a segment of Drosophila DNA called cDM 356. This DNA fragment contains a 3000 bp tandem repeat and is homologous to regions of the chromosomal terminus. This cloned DNA fragment may contain the elements responsible for transposition of the dispersed repeated gene families to new chromosomal sites.

An interesting aspect of the copia gene is its relationship to the chromosomal terminus. In Drosophila, the chromosomal termini consist, in part, of the tandemly repeated DNA segment of 3000 bp and a copy of the copia gene. All of the 3000 bp repeats observed contain a region of homology which is partially homologous to the terminal redundancy of the genes of the copia family. Furthermore, the location of this region of homology within the 3000 bp repeat corresponds to the site in the tandem repeat where the copia gene is inserted. These observations suggest that the copia gene is inserted in the 3000 bp repeat by a specific reciprocal recombination event as predicted by Shapiro's transposition mechanism (See Section B-1 above).

Elements which change their location within the genome will affect gene expression of other genes as long as the movement of such elements is not restricted to a small number of genetically inert sites. The elements of Drosophila dispersed repeated gene families do not appear to be limited in this way, and over 100 sites for each of these families have been

observed. Elements of the dispersed repeated gene families might promote chromosomal translocations, inversions, and deletions by serving as "portable regions of homology" between otherwise non-homologous regions. Two elements acting in concert might be able to transpose a larger segment of DNA located between them. It may be that the sole function of these elements is to provide genetic variability, and their gene products may only be necessary for the maintenance and mobility of the elements themselves, rather than for any other cellular purposes.

Chromosome instability is closely coupled with and may be a causative factor in the transformation of cells to the malignant state. Transposable elements are one of the major promoters of chromosome instability in prokaryotes and may play a similar role in eukaryotes. The work of Rubin, in demonstrating the existence of transposons in Drosophila, may now allow a study of the structure and mode of action of eukaryotic transposable elements at the molecular level.

C. Ribosome Structure and Function

GM 19843 (Charles Cantor) Columbia University
GM 24763 (Walter Hill) University of Montana
GM 26202 (Joe Johnson) Atlanta University Center
GM 22150 (Lawrence Kahan) University of Wisconsin
GM 24034 (James Lake) University of California, L.A.
GM 15399 (Umadas Maitra) Yeshiva University
GM 20427 (Maysayasu Nomura) University of Wisconsin
GM 06965 (Jean-Paul Revel) California Institute of Technology
GM 20277 (David Sabatini) New York University
GM 17924 (Robert Traut) University of California, Davis

The ribosome is the universally distributed cellular organelle which is the unique site of protein synthesis; and the final step in the expression of the genetic code leading to formation of products used in metabolism, cell structure and growth. The ribosome, whether in lower or higher organisms, is complex and contains three or four different RNA molecules and from fifty to seventy different protein molecules. The polymerization of activated amino acids into a protein would seem a simple enough reaction, unlikely to require very complex enzymatic machinery, until one considers the need for a high degree of specificity in polymerizing the amino acids precisely in the correct order. Many genetic diseases result from the presence of only one incorrect amino acid in a protein. If the ribosome did not have an extremely low error rate, the results would be disastrous to cells. The surprising complexity of the ribosome is probably related to the problem of maintaining perfect specificity or fidelity in the translation of the genetic code.

Certain aspects of ribosomal behavior have been studied intensively with NIGMS support. Three areas in particular show significant gains and promise of exciting expansion in knowledge. These areas are: information

concerning the topography of the ribosome; evidence concerning the degree of complexity in essential components and the cooperativity required among the components; and preliminary information concerning the difference between prokaryotic and eukaryotic ribosomes. The latter has implications in control of disease through design of antibiotics and in the study of control of bacterial viruses.

The biochemical composition of ribosomes from various sources has been well characterized. The smaller ribosomal subunit from E. coli has a molecular weight of approximately 1.0×10^6 Daltons. This subunit consists of 16S RNA and 20-21 different proteins. The proteins are small and have been purified and partially or completely sequenced. Nomura and others have shown that the small subunit is a self-assembling system. Under appropriate ionic and temperature conditions subunits can be reconstituted from 16S RNA and the constituent proteins into fully functional particles. The assembly process proceeds step-wise, first requiring the presence of a small number of proteins. After these proteins have been incorporated, subsequent proteins participate in the assembly reaction. The information has been codified into an "assembly map" for E. coli denoting the sequence of protein additions during reconstitution. Fully functional hybrid subunits can be reconstituted from such diverse species as E. coli and B. Stearothermophilus, which suggests that a model based on studies of E. coli may be applicable to all prokaryotic subunits.

The larger ribosomal subunit from E. coli has a molecular wieght of approximately 1.55×10^6 Daltons. This subunit consists of a 5S and a 23S RNA and 34 different proteins which are quite small (10,000-30,000 Daltons). All proteins have been purified, many have been partially sequenced and several 50S proteins, including L7 and L12, have been completely sequenced.

The large subunit from B. Stearothermophilus is a self-assemblying system which has been successfully reconstituted by Nomura. Reconstitution of E. coli large subunits has also been reported.

1. Topography of the Ribosome

Lake altered the perception of ribosomal structure significantly by describing the topography of the E. coli ribosome at high resolution. First, Lake modified standard electron microscopic negative staining procedures to achieve high resolution (2-4 nm) images of three dimensional structure which indicated elaborate contours. Lake then collaborated with Kahan and Nomura to determine the location of several individual proteins and specific regions of RNA on the ribosome by utilizing antibodies directed against large and small subunit ribosomal proteins and against specific modified nucleotides. Also, antibodies directed against the initiation factors, 1F1, 1F2 and IF3 and against the elongation factors EF-Tu and EF-G were used to map their locations on initiation complexes and on ribosomes in the appropriate functional states. They further intend to study the path of messenger RNA through the ribosome and antibiotic binding sites by this immune electron microscopic method.

Kahn prepared highly specific antibodies against all the 30S proteins of E. coli and antibodies toward the B. Stearothermophilus functional equivalents as controls. Antibodies have been prepared by Nomura and others against all 50S proteins except L8 and L9. Utilizing these antibodies, Lake and his collaborators have achieved preliminary locations for all 21 small subunit proteins and for approximately one quarter of the large subunit proteins to date. Also, orientations of the subunits within the 70S ribosome have been determined. As a result of these protein mappings, important functional regions identified include the codonanticodon interaction site, the tRNA binding sites, initiation factor binding sites, elongation factors binding sites, and the site of peptidyl transferase.

The location of functional regions as described by Lake and his collaborators has provided clues to some of the molecular events that occur during the initiation and elongation cycles of protein synthesis. For example, for some time it has been shown that the elongation phase of protein synthesis requires several steps which occur on separate but neighboring sites of the ribosome. During the elongation cycle, a peptidyl tRNA bound to the ribosome at the "P" (peptidyl) site is joined, through the action of a transferase (the transferase site), to the N-terminus of an amino acyl tRNA bound at the "A" (amino acyl) site, resulting in an elongated peptidyl tRNA. Following elongation, the new peptidyl tRNA is translocated back to the "P" site, permitting a new cycle to begin. Having mapped the location of the "P", transferase, and "A" sites to contiguous regions on the surface of the small subunit in E. coli ribosomes, another binding region was discovered near the "A", transferase, and "P" sites, extending this total functional region on the ribosome. This new binding region has been designated the "R" (recognition) site. In Lake's hypothesis, it is thought that this "R" site may serve as a proofreader to permit discrimination of any amino acid-tRNA complex in which the tRNA has a codon error; this supplies a mechanism for an incorrect complex to be ejected.

In collaboration with Sabatini and others, Lake has begun studying the eukaryotic ribosome. The binding site for the eukaryotic initiation factor eIF-3 on the small subunit has been mapped.

2. Functional Studies and Cooperativity of the Components

In addition to the microscopic approaches describing the topography of the ribosome, complementary biochemical and physical approaches are being utilized to improve our understanding of the functional organization of the organelle. One such approach is to use bifunctional cross-linking reagents to measure distances between nearest neighbor ribosmal proteins. All 30S and many 50S proteins have been shown to be accessible to chemical modification, including these cross-linking reagents. Experiments using bifunctional reagents to measure distances have traditionally been calculated assuming spherical protein units, however, it is now understood that some ribosomal proteins have an elongated shape in the functional state on the surface of the ribosome.

One researcher who has been particularly productive in using bifunctional cross-linking reagents to identify nearest neighbors is Traut. Traut has focused on groups of proteins which, though they may not be contiguous, reside at certain interesting active sites. In general, the agreement between antibody mapping and cross-linking studies of ribosomal proteins has been extremely good, and the results obtained by both techniques have provided information that would not be presently available using either technique alone. For example, as a result of comparing the results of cross-linking 1F1, F2, and 1F3 to small subunits with an antibody mapping of S5, S11, S13, and S19, a binding site for fmet-tRNA has been suggested.

Both reconstitution studies, which showed that nearly every component of the ribosome is essential for proper function, and mapping studies, which revealed highly irregular contours and suggested a variety of functional sites, have indicated the complexity of the ribosome. Since it is unlikely that every component of a ribosome, though essential, is located directly at a functionally active site, it has become appreciated that the ribosome must be a highly cooperative entity whose structure is maintained by a high degree of interaction between the individual components.

A variety of techniques are being developed to explore functional cooperativity, including deletion of components, antibody association, and chemical modification. One investigator who has shown considerable ingenuity in studying cooperative interactions is Johnson, who has attached fluorescent probes to selected proteins in the E. coli ribosome. After exposure to light, the probe emits light in varying amounts depending upon the degree of freedom of motion of the tagged molecule. (If a protein is tightly bound, less emission will occur.) Such studies can be used to assign binding sites to various factors involved in protein synthesis, or in a more sophisticated manner, to study conformational states of the ribosomal complex. Fluorescent spectroscopy is an excellent technique for following conformational changes because of its sensitivity and because the signal can be monitored nondestructively and continuously. In addition, distance changes over the range of 2-8 nm can be measured. Fluorescence spectroscopy can demonstrate the existence of a conformational change, determine the magnitude of the change, and measure the kinetics of that change. Johnson has been particularly ingenious in approaching the question of ribosome function through the tagging of tRNA. TRNA is intimately involved in all primary ribosomal functions, whereas other components of the system only participate in some of the reactions.

Johnson has prepared several fluorescent-labeled tRNAs which will allow him to determine the kinetics and energetics. It is likely that the most interesting and significant results obtained in the next few years will be in the area of confirmation and characterization of conformational changes.

3. Eukaryotic Studies

This Institute has supported the work of several important groups of investigators who have advanced the knowledge of ribosomes to a point at which the more complex eukaryotic ribosome may be studied. Several groups including that of Maitra have explored the role of initiation

factors which associate with ribosomes in protein synthesis. Maitra has embarked on studies of the initiation of protein synthesis in mammalian systems with particular emphasis on those partial reactions which are unique to the eukaryotic system. Using calf-liver as a source of isolation of initiation factors, he has isolated a new protein factor that regulates GTP-dependent formation of a ternary complex by the eukaryotic initiation factor eIF-2. This complex, eIF-2 GTP Met-tRNA is believed to be the first unique step in initiation of protein synthesis in eukaryotic systems. Imitation of protein synthesis is most likely a key point of control of translation in mammalian systems.

D. Gene Regulation

1. Regulatory Mechanisms in Trytophan Biosynthesis
GM 22131 (Ronald Somerville) Purdue University

One of the aspects of molecular genetics supported heavily by NIGMS is the regulation of gene expression. It is generally agreed that regulatory phenomena will be properly understood only when the physico-chemical principles behind the interaction of proteins with specific target regions of nucleic acids are elucidated. Examples of some important protein-nucleic acid interactions are: 1) the recognition by RNA polymerases of sites in DNA for transcription initiation (promoters), 2) the recognition by regulatory proteins of control sites in DNA (operators), 3) the recognition by certain proteins of sites in DNA for chemical cleavage (as, for instance, with site-specific recombination or site-specific restriction endonucleases), 4) the repair of DNA by enzyme systems which act to reverse mutagenic lesions, 5) the processing of RNA either to remove internal non-coding sequences or to modify sequences at each extremity appropriate for optimal translation, and 6) modification of sugars and bases in rRNA and tRNA to prepare these molecules for their part in gene expression.

At the nucleotide level, there are few concise and universal definitions of what constitutes a given class of recognition sites. Wide variations in structure are seen even when one compares those promoters capable of being recognized by the RNA polymerase of Escherichia coli. One suspects that promoter recognition involves extreme flexibility on the part of RNA polymerase, that some promoter sequences have a high probability of dissociating from polymerase before transcription can ensue, and that protein-DNA interaction parameters will tell us something fundamental about the "strength" of a given promoter.

One approach to the dissection of this problem is to assemble a set of DNA sequences differing from one another in a known way (for example, through specific single base pair differences), that in addition may or may not manifest promoter activity. This has been the approach chosen by Somerville. Obtaining such a set of homologous DNA sequences has until recently been a formidable problem. However, the identification of several regions of DNA

which can be converted by mutation to strong, constitutive promoters has greatly simplified matters because such matters result in easily scored phenotypes. These DNA regions are part of the sequence coding for one of the five proteins involved in tryptophan biosynthesis.

At least 12 DNA regions within the trp biosynthetic operon have been found that are normally inactive as promoters, yet can mutate to promoters via a single base pair change. The most intensely studied of these lies in the trpD gene and is known as trpD11. Somerville has found that a change in the DNA of a particular codon from CAA to TAA, an ochre nonsense mutation, created a non-regulated, high efficiency promoter of mRNA synthesis from a "down-stream" trp operon gene. By a variety of procedures, a collection of 20 different, mutationally-connected changes at the trpD11 codon have been obtained. Somerville found that changes in the second or third base pair, do not affect promoter activity, whereas a change in the initial base pair does affect promoter activity. These results demonstrate the profound influence that a change in nucleotide sequence in the neighborhood of a potential promoter may have on its expression. They also demonstrate in a simple and direct fashion that the RNA polymerase displays a less-than-rigorous specificity with regard to the DNA sequences that are recognized as transcriptional start sites.

The availability of promoter mutations with specific, readily scorable nutritional phenotypes has opened up a broad area of investigation. Within a few years it should be possible to identify by mutation each of the elements required for productive recognition of a given promoter. For example, it is already established that certain types of missense mutation within the subunit of RNA polymerase lower the efficiency of recognition of the trpD11 promoter, without observable effects on the potency of other, unrelated promoters. The ready applicability of new techniques in the area of DNA sequence determination will deepen our understanding of the structural basis for transcription and the control of transcription.

2. Molecular Approach to Fungal Dimorphism
GM 18293 (Paul Sypherd) University of California

A wide range of taxonomic groups of fungi possess the ability to grow either as mycelia or as yeast. The physiological control of this dimorphism varies between organisms, but is often regulated by temperature, medium, or the nature of the gas in which the organism grows. The range of fungi exhibiting dimorphism includes both saprophytic and pathogenic forms. Certainly, most of the more pathogenic fungi exhibit the property of dimorphism.

For the past five years Sypherd has studied the mechanisms for the regulation of gene expression in Mucor racemosus. This fungus offers the advantage of low pathogenicity, rapid and predictable growth in yeast or mycelial form in laboratory media, and the availability of a genetic system. Early experiments were concerned with determining a number of biochemical correlates of the yeast-to-hypha transition. For example, they showed that

cyclic AMP is involved in regulating growth in the yeast phase and that a volatile pheromone regulates anaerobic hyphal development. Sypherd has also studied the control of catabolic enzymes, the expression of the isozymes of pyruvate dehydrogenase, and the flux of carbon through several glucose catabolizing pathways. More recently he has studied alterations in the translational system which occur when yeast forms are induced to develop into hyphae. For example, Sypherd discovered that after the cells received an environmental signal to undergo morphogenesis, there was a dramatic stimulation in the differential rate of protein synthesis, and that this stimulation resulted from an increased rate of movement of ribosomes along messenger RNA. These studies showed that amino acids were polymerized into peptide bonds at the rate of two amino acids per second per ribosome in the yeast phase with an increase in this rate to eight amino acids per second per ribosome at the maximum in the hyphal phase.

Ribosomes of the fungus were analyzed to see if changes in a component of the translational system could be detected. A single protein (S-6) of the small ribosomal subunit was shown to undergo dynamic phosphorylation during fungal morphogenesis. That is, cells in the yeast phase had an average of less than one phosphate per molecule of S-6, while cells which were rapidly producing "germ tubes" were maximally phosphorylated with 3 phosphates per molecule of S-6 protein. Thus, during the time that the rate of ribosome movement is accelerating, so is the rate of phosphorylation of the single ribosomal protein. Presently, Sypherd is attempting to develop an in vitro system for protein synthesis which would allow him to test each of the components of the translational system during each of the fungal phases.

Recently, Sypherd has focused on the regulation of individual proteins as they might be affected during the course of morphogenesis. First, he has determined the effect of morphogenesis on the activity of specific enzymes and has found two, the NAD-dependent glutamate dehydrogenase and ornithine decarboxylase, which are synthesized almost specifically during hyphal growth. He is continuing his studies into the details of the regulation of these enzyme systems. Secondly, two dimensional gel electrophoresis has displayed 400 to 500 gene products. Twelve proteins are specific to the yeast phase and another 12 or so proteins are specific to the hyphal phase. Sypherd has also discovered a number of proteins which are regulated quantitatively during morphogenesis. By the use of a wheat germ in vitro system, it has been shown that some of these morphology-specific proteins are regulated at the level of translation, by isolating the specific messenger RNA at times when a specific gene product cannot be found in vivo. These represent nontranslated or quiescent mRNAs. The mechanism whereby mRNAs can exist in cells and not undergo translation will, hopefully, be determined.

During gene regulation experiments, which involved two-dimensional gel electrophoresis, a major cell protein which undergoes post-translational modification by way of methylation was discovered. The effective activity of this protein, which is about the eighth most abundant protein in yeast, and about the fourth most abundant protein in hyphae, is regulated

both by an increase in its specific rate of synthesis and also by an increase in its level of methylation. The protein, designated "MMP" for major methylated protein, is associated with the wall-membrane fraction of both yeast and hyphal cells and is labeled very lightly when whole cells are incubated with lactoperoxidase and I^{125}. This suggests that the protein is a surface or membrane protein. Because of its unique properties (molecular weight 55,000 and pI 9.7) and its abundance in the cells, Sypherd examined other fungi for its presence. This same protein as judged by its physical properties and methylation, is found in Candida albicans, Saccharomyces cerevisiae, and Neurospora crassa.

With the apparent increase in mycotic infections in North America, it is hoped that these results, obtained at a very basic level of cellular biochemistry, will provide useful approaches for studying the pathogenesis of fungi responsible for substantial morbidity and mortality in man. As various forms of therapy involving the use of immunosuppressants increase, so has the incidence of infections due to opportunistic fungi. Many of these, such as Mucor and Candida, are dimorphic, and there is considerable lore that the dimorphism is indeed a feature of invasiveness per se. The nature of the patients who experience such infections is also important. Very often such patients are immunocompromised either through the use of heavy radiation or through the use of immunosuppressant chemotherapeutic agents. In such cases, the early detection of mycotic infections is very important, since it is not possible to employ the highly toxic drug Amphotericin B routinely in the maintenance of such patients. With these problems in mind, Sypherd has begun a study of the control of gene expression in Candida albicans. Preliminary experiments have shown that these organisms contain the major methylated protein, and in addition that there are proteins which are specific to the yeast phase and to the "germ tube" phase. Germ tubes are formed in this organism when yeasts, also called blastosphores, are placed in a suitable medium which allows them to germinate. In collaboration with Edwards, UCLA, Sypherd has begun a study of the role of germ tube formation in the establishment of hematogenous infections in experimental animals. Edward's preliminary experiments have shown that mutants of C. albicans which do not produce germ tubes, also fail to establish infection in this animal model. It is their aim to use the animal model of Candidiasis to study the role of germ tube formation and of the germ tube-specific proteins seen on two-dimensional gels in establishment of the infection. One hopeful result of such studies will be the development of sero-diagnostic tools which will make it possible to detect Candida infections at very early stages.

3. The Role of Histones in Embryonic Development
GM 24019 (Leonard Cohen) Institute for Cancer Research

Cohen and his colleagues at the Fox Chase Institute for Cancer Research are engaged in studies aimed at the elucidation of the functions of chromosomal proteins, particularly their role in the generation of cellular diversity during development.

Embryonic development involves a progressive increase in phenotypic variety among cells containing the same genetic information. The differentiation of multiple cell types must depend upon selective modulation of gene expression. A prominent feature of early development is a transition from a uniform high rate of cell division to lower variable division rates. It seems probable that the modulation of both gene expression and division rate involves the protein components of chromatin.

The predominant structural proteins of chromatin are the histones, which are intimately associated with the DNA. Four histones, H2A, H2B, H3, and H4, are believed to be responsible for the basic conformation of DNA. The four histones associate with one another to constitute an oligomer around which the DNA is coiled. The complex of this oligomer with DNA gives rise to a repeating structure, variously termed core particle, nu body, or nucleosome. The oligomer has been considered to be relatively invariant in phylogeny and totally invariant in ontogeny, although current work indicates considerably more variation than was generally believed.

In contrast to the four histones forming the oligomer, the other major histone, H1, is known to be heterogeneous and highly variable in evolution. Although distributed throughout the genome, H1 proteins are not part of the nucleosomal oligomers. They may provide structural variety of nucleosome chains, and may also cross-link chromatin fibers.

The work of Cohen and others has revealed that several subtypes of each of the histones are synthesized during embryonic development. In the sea urchin, the subtypes, designated α, β, γ, and δ, are synthesized at specific embryonic stages. A series of such switches in histone synthesis has been demonstrated by non-ionic detergent electrophoresis, a method which discriminates proteins differing in hydrophobic properties, even though very similar in molecular weight and ionic charge.

Following the designation of the α to δ histone subtypes, three additional "histone-like proteins" were identified as principal constituents of chromatin during very early cleavage. Since these proteins resemble histones H1, H2, and H2B, and since no other subtypes were found at such an early stage, it is concluded that these proteins are probably cleavage subtypes of the three histone classes. They were designated CS1, CS2A, and CS2B.

Within each of the three histone classes, CS subtypes are synthesized from fertilization through early cleavage, and α subtypes are synthesized from the second S-phase after fertilization until the blastula stage. These are the only histone components produced in the early cleavage stages, and their synthesis is completely shut off by the beginning of gastrulation. The synthesis of seven other subtypes of H1, H2A, and H2B, begins later, each at a specific time between the 16-cell and blastula stages. They have been termed β, γ, or δ, according to the time at which their synthesis is first detectable. These are the only histone classes synthesized after the beginning of gastrulation, and their synthesis continues until the prism stage.

During the past year, Cohen's efforts have been focused on: 1) the mechanisms controlling the early switches in the synthesis of histone subtypes, 2) the question of whether there are additional switches in later development, and 3) differences between histone subtypes with respect to the important function of phosphorylation.

1. Switch mechanisms Cohen's group has shown that the unfertilized egg contains mRNAs for all of the cleavage stage (CS) and α histone subtypes, as well as a program for the selective translation of $mRNA_{CS}$ until the third round of replication, and the subsequent translation of mRNA α. They now report the interesting finding that these mRNA subtypes are destroyed after their translation ceases. The selective destruction of the particular mRNAs renders those off-switches irreversible as long as the genes coding for the subtypes remain inactive. One exception may be CS2B, since a protein with the electrophoretic mobility of this subtype was found among the products of in vitro translation of gastrula mRNA.

2. Histone subtypes synthesized after gastrulation. It has been found that the late larva (rudiment stage) of the sea urchin does not synthesize CS or α subtypes, with the exception of one protein which might be CS2B. Furthermore, neither the sperm nor the mature lining cells of adult animals were found to contain any detectable CS or α subtypes, again with the exception of a protein resembling CS2B. Thus it is likely that most of the early histone subtypes, once switched off, are never switched on again until oogenesis. The question of whether CS2B is an exception to this conclusion will require further work.

3. Phosphorylation differences between histone subtypes. Drastic differences in phosphorylation rate have been found between histone subtypes. When gastrulae were incubated with ^{32}P-phosphate, CS1 took up at least 20 times more label than did H1α, while H1β and H1 α were not detectably labeled. Similarly, CS2A was labeled extensively, but the α, β and γ subtypes of H2A were not. The results show that the CS subtypes are functionally distinct from other subtypes, or are found in cells that are dividing more rapidly than cells containing the other subtypes, or both. This view is in accord with the fact that the rate of cell division is extremely rapid when CS subtypes are the principal histone constituents of chromatin, less rapid when α subtypes become incorporated into the chromatin, and least rapid when β, γ, and δ subtypes begin to be incorporated. The properties of the histone subtypes in chromatin indicate that at each stage of development, cell division rate is correlated with the nature of the histone subtypes being synthesized.

The discovery of switches in synthesis among multiple histone subtypes provides evidence that histones may play a fundamental role in the developmental process, and presents the possibility of investigating the nature of that role. These studies provide specific instances of mechanisms controlling the expression of specific genes (the histone genes) at the levels of both transcriptions and translation. Analysis of these phenomena should clarify some of the basic processes in development and cell differentiation.

4. Gene Regulation and Chromosome Number
GM 22103 (Robert Krooth) Columbia University

About one human pregnancy in six terminates in a spontaneous abortion and between one half and one third of the aborted fetuses have been shown to have an abnormal number of chromosomes. Why are abnormal chromosome numbers lethal even when all chromosomes are present in the cell at least once? To answer this question Krooth and collaborators have studied fibroblast cultures grown from spontaneous abortuses collected from medical research laboratories throughout the world as well as from the Cell Repository at the Institute for Medical Research in Camden, New Jersey. Most of the cell cultures were found to have only one extra chromosome and only those were included in the study. Overall the collection presently includes trisomies of about half of all human chromosomes.

The investigators asked whether fibroblasts with extra chromosomes make products not found in normal fibroblasts. In particular, can they make products not normally found in fibroblasts but present in other differentiated cells? So far, Krooth has demonstrated that fibroblasts with an extra Chromosome 2 produce acetylcholinesterase, an enzyme not found in normal fibroblasts or in fibroblasts with trisomies of other chromosomes.

Although this is a relatively preliminary result, it has important implications. The hypothesis Krooth presents to explain the findings is that the turning-on of acetylcholinesterase production is the result of an escape of the gene for acetylcholinesterase from its normal control. The presence of three copies of Chromosome 2 produces an imbalance in the cell that results in the activation of genes normally repressed in fibroblasts. Since the mechanism of gene control in eukaryotes is very poorly understood, the approach chosen by Krooth may provide some insight into how it works.

Abnormal chromosome numbers are found not only in aborted fetuses but also in such conditions as birth defects, mongolism and cancer cells. A better understanding of what happens in a cell with unbalanced chromosomes may therefore have important implications for human disease.

5. Possible Genetic Basis for Antibody Diversity
GM 09541 (Walter Gilbert) Harvard University

The binding site of an immunoglobulin molecule is a pocket between its two subunits, a light and a heavy chain. The part of the light chain in

contact with the antigen, the amino terminal region termed the variable region, differs extensively from one immunoglobulin molecule to the next. Although different, the variable regions do, however, show certain consistencies in structure. In the mouse this structure consists of three hypervariable regions of five to ten amino acids each around positions 30, 55, and 90 in the immunoglobulin chain. In the three dimensional structure, these hypervariable regions are the specific segments of the polypeptide chain that are in contact with the antigen. Immunoglobulin light chains fall into subgroups, based on the sequence of amino acids in each hypervariable region. A similar description can be presented for the immunoglobulin heavy chain.

The question which arises from recognition of these facts and which has plagued immunobiologists for decades is how this antibody diversity arises from the cell's genetic structure.

Recent work by an NIGMS grantee may aid in answering the question. Working with the embryonic light chain gene from the mouse, Gilbert used recombinant DNA techniques to isolate and clone the light chain variable region DNA. From a comparison of the sequences of immunoglobulins from two subsets of light chains, Gilbert has demonstrated three hypervariable sequences set in a framework of the remainder of the variable region. That is, base pair substitutions leading to amino acid changes are confined to three regions of the gene. Furthermore, Gilbert found there are certain regions of sequence homology between the three hypervariable gene regions, and he has identified the particular base pairs within them which could be altered to yield the known amino acid substitutions.

Although some of the molecular characteristics of the hypervariable gene regions are now clear, there are no features so clear as to define the mechanism by which these regions become more labile to base changes than the rest of the genome. The partial sequence homology, however, may suggest that there are regions that could serve as a recognition site for an enzyme that could cleave or modify the DNA to make the sequence labile. In his study of the basis of the lability, Gilbert analyzed the sequence of the whole mouse light chain gene. There are several sequences of nucleotides which do not appear to be expressed in protein. These sequences Gilbert calls introns. For contrast, the sequences that are expressed are termed exons. His analysis of the mouse immunoglobulin light chain gene shows the following DNA structure: leader (45 base pairs) - intron (93 base pairs) - variable (306 base pairs) - intron (1250 base pairs) - constant (348 base pairs). Thus, the gene has large amounts of untranslated DNA, the introns. This intron-exon structure may have highly significant implications for understanding genetic variability, particularly for immunoglobulins. Gilbert suggests some possible molecular mechanisms based on the gene structure he has described.

First, the RNA transcript of an intronic region may contain some secondary structure which could serve as signals for enzymes, such as those that excise tRNA's from their precursors, to cut out a section. The cut could be resealed by an RNA ligase.

Second, single base changes could change not only protein sequences but, if they occur near an excision boundary, might also change the splicing patterns, resulting in deletion or addition of whole amino acid sequences. Thus, single rare mutations can generate novel proteins more rapidly than single mutations in the exon alone can produce.

Third, even mutations in silent third base positions could alter the secondary structure of the RNA transcript and thus the joining, so that the modified message may be a mixture of the old and new gene product. Thus, Gilbert points out, the genetic material would not have to duplicate to provide a mutant copy of an essential gene.

Fourthly, Gilbert postulates that recombination is facilitated by the extra space which introns provide within a gene. In addition, specific recombination between introns could bring together exons into a new transcription unit to make specially altered proteins such as immunoglobulins or differentiation proteins. Such alteration might occur by recombination within or transposition of intron (DNA) segments, or alternatively it may arise from production of a new enzyme that could change the processing of the RNA message.

E. Medical Applications

1. Fetal Alcohol Syndrome
GM 15253 (Arno Motulsky - Hall) University of Washington

Human birth defects can be caused by genetic abnormalities (such as chromosomal aberrations or single gene defects), by environmental agents, and most frequently probably by subtle interaction of genetic and environmental causes. The differentiation of genetic birth defects from clearly environmentally caused malformations is particularly important for genetic counseling.

The research efforts of several investigators of the Genetics Center at the University of Washington have been directed at identification of human birth defects caused by previously unsuspected environmental agents such as alcohol and hyperthermia and by drugs such as dilantin and coumadin. The Seattle investigators have excelled in the identification of recognizable syndromes produced by specific agents. Large collaborative studies in the past have failed to recognize these subtle syndromes since their identification requires clinicians expert in the evaluation of infants with birth defects and alert to minor dysmorphologic aberrations.

Such work has led to the unequivocal identification of the fetal alcohol syndrome in babies of mothers with relatively heavy chronic alcohol intake (>90 ml of ethanol per day). This syndrome manifests with small head size, a shortened width of the eyelid space, thinning of the red portion of the upper lip, and loss of definition of the vertical groove above the midregion of the upper lip. A variety of other morphologic abnormalities are frequently seen in addition to mild to moderate mental retardation and growth deficiency.

It has also become apparent that more moderate alcohol intake can have an impact on morphogenesis with reduced birth size and weight and some mental retardation. There is a correlation between the severity of the physical aberration and the degree of mental retardation. The frequency of the fetal alcohol syndrome is estimated to be two live births per 1000 with partial expression occurring as frequently as between 3-5 live births/1000.

These data indicating that alcohol is a potent teratogenic agent in man, have wide-ranging sociocultural and economic implications. Alcohol appears to be a major avoidable environmental agent implicated in the etiology of mental retardation.

2. Origin of Tumor Cells in Man
GM 15253 (Arno Motulsky - Fialkow) University of Washington

The exact origin of human cancer is rarely known. However, genetically labelled cells can be utilized to study whether a tumor arose from a single cell or whether many cells contributed to its origin. Knowledge regarding this point may be useful in elucidating the cause of the tumors and devising rational strategies for treatment.

Fialkow has utilized the phenomenon of human X-chromosome dimorphism to study the origin of tumor cells. All human females have two X chromosomes - one of maternal and one of paternal origin - in each of their body cells. Only one of the X chromosomes in a given body cell is functionally active and and, on the average, 50% of the cells in a female function with only the maternal X chromosome and 50% with only the paternal X-chromosome active. Thus, if the maternal and paternal X chromosomes are labelled with different alleles of the same gene, one can identify which chromosome is active in a particular cell in heterozygous individuals. The enzyme glucose-6-phosphate dehydrogenase (G6PD) exists in two easily distinguished forms in man, both of which have normal function. These are known as the G6PD A and G6PD B enzyme, respectively. Studies on tumor origin can therefore be carried out on tumor-bearing women who have inherited the G6PD A gene from one parent and the G6PD B gene from the other parent. A single normal cell will either be of type A or of type B. A mixture of normal cells will show both types. A tumor of unicellular origin would be expected to contain either the A enzyme or the B enzyme but not both enzymes, whereas a tumor of multicellular origin would show the normal pattern of both the A and B enzymes.

Using this system, Fialkow demonstrated that many human tumors have originated from a single cell. These findings are compatible with the suggestion that a tumor develops as a result of a rare genetic accident in a single cell such as somatic mutation.

The implications of the origin of a neoplasm from a single cell are well illustrated by studies on chronic granulocytic leukemia. All the leukemic cells in a single individual appear to be descended from a single progenitor - a finding compatible with the mutation hypothesis for the origin of this type

of leukemia. G6PD studies show that this disease also affects other types of white blood cells as well as red blood cells and platelets, even though the clinical manifestations primarily involve the granulocytic white blood cells. Thus, this type of leukemia begins in a primordial stem cell and all differentiated descendants of this stem cell carry the original leukemia-causing mutation.

Polycythemia vera, a disease characterized by excessive proliferation of red blood cell precursors, also appears to arise from a single stem cell for granulocytes, red blood cells and platelets, even though the predominant clinical manifestations only involve the red blood cell. The G6PD marker studies indicate that the basic abnormality is intrinsic to the stem cells.

Are there any normal stem cells left in chronic granulocytic leukemia or polycythemia vera? Unless normal stem cells are present, treatment designed to "kill" all abnormal stem cells will, if successful, also kill the patient. Using G6PD as a marker, residual normal stem cells in polycythemia vera could be demonstrated but their expression is suppressed. The demonstration of residual normal stem cells suggests an alternate means of treatment to chemotherapy designed to kill the abnormal cells. One can search for ways to stimulate the suppressed normal stem cells so that they can repopulate the blood cell system and hopefully "crowd out" the abnormal stem cells. In contrast to polycythemia vera, there is no evidence for residual normal stem cells in chronic granulocytic leukemia. This difference between the two diseases indicates that the basic abnormalities are very dissimilar although both diseases may involve the same stem cell.

Various studies with the G6PD technique of the putative viral malignancy Burkitt's lymphoma have shown this tumor to be a unifocal disease which begins with a single cell and spreads throughout the body. Late recurrences of the tumor following treatment are not related to the original tumor. These findings have important implications for therapy since suppression of the major tumor line alone will not cure the patient.

The G6PD typing technique has been extensively used by Fialkow for the study of many different tumors. It has elucidated the origin of certain hereditary and endocrine tumors which are multicellular in origin. The somatic mutation theory therefore does not apply to all tumors.

3. Mechanism of Methotrexate Resistance in Mammalian Cells
GM 14931 (Robert Schimke) Stanford University

Methotrexate is a cancer chemotherapeutic agent. After a certain period of exposure to methotrexate, cancer patients become resistant to its action, a phenomenon that can also be demonstrated in cell culture. Schimke has been studying the mechanism of cellular resistance to methotrexate at the molecular level. Although this work was supported largely by the Cancer Institute, the molecular genetic techniques were concurrently developed and refined by Schimke under NIGMS support.

The investigators found that a number of methotrexate-resistant cultured cell variants of both hamster and mouse origin have become resistant because of an increased rate of synthesis of a specific enzyme, dihyrofolate reductase. The cause for the increased enzyme synthesis (up to 300 fold) was traced to a selective amplification of the dihydrofolate reductase genes. While in some cell variants the high gene copy number is unstable in the absence of methotrexate, i.e., the cells lose the high gene number and the high resistance, in other cell variants the amplified genes are stable. This mechanism may well be occurring in the case of human malignancies, where resistance is often associated with elevated dihydrofolate reductase levels. It is also interesting to note that when patients stop receiving methothrexate, their malignancies often again become sensitive. The mechanism of the development of methotrexate resistance in cultured cells is strikingly similar to many instances of antibiotic resistance in bacteria, suggesting that similar molecular events may be involved (See Section B, above).

F. New Methodology

A Strategy for the Characterization of the Human Genome
GM 24030 (Leonard Lerman) State University of New York at Albany

Advances in scientific disciplines have often been dependent on the development of appropriate methodology. It is the thesis of this highlight that we are now in the midst of a period of advances in the technology for purification, separation and sequencing of nucleic acids. These advances have close parallels to the similar advances made in the peptide and protein field in the period from about 1935 to 1970. For example, the purification, studies of specificity, and the use in protein structure determination of proteolytic enzymes such as trypsin and chymotrypsin parallels the more recent experience with restriction enzymes for nucleic acids. The analysis of amino acids and peptides (Moore and Stein) corresponds to similar advances on the analysis of nucleic acid building blocks by Edwin Chargaff and Waldo Cohn. The development of sequencing methods for peptides and proteins (Sanger, Edman) has its counterpart in the work of Holley, and Gilbert and Sanger. Perhaps the one revolutionary tool in the nucleic acid area that does not have a strict counterpart in the protein area is recombinant DNA technology. This is basically a tool for (1) "amplification" of specific sequences and for (2) transcription and translation of these sequences in a "foreign" environment. The "amplification" aspect of this technology has some correspondence to protein purification, but is far more powerful.

Geneticists have been most interested in two-dimensional electrophoretic separation methods in gels. In the protein area, such methods were fairly recently developed by O'Farrell (1975). Such methodology, unlike most others, has the advantage of displaying all of the soluble proteins of an organism in a two-dimensional array. Hundreds, and sometimes more than one thousand protein spots can be visualized. This type of display is,

of course, of particular interest to geneticists, since it permits them to seek to correlate genetic or functional differences between two similar organisms with possible differences in their gene products, the proteins.

A similar development in the DNA area to the O'Farrell technique would be more difficult. What would be examined in such a technique would not, in general, be whole DNA molecules, which are huge, but rather restriction enzyme fragments which, very approximately, correspond in size and informational content to genes or proteins. The number of fragments expected and thus the degree of resolution required increase by a factor of 100 between the bacterial virus λ and the bacterium E. coli, by another factor of 10 between E. coli and Drosophila, and a factor of about 100 between Drosophila and man. Three to five restriction fragments are expected for λ, a few hundred would be expected for E. coli, but hundreds of thousands of spots would be expected in the human genome.

Lerman has been able to display about 350 DNA fragments, resulting from the Eco R1 restriction enzyme digestion of E. coli DNA, on a two-dimensional gel. The sensitivity of the technique is such that four additional fragments can be seen in the pattern after the E. coli has been lysogenized with the virus λ.

Lerman's work hints at the possibility of inferring the genotype of an organism from the DNA of an organism, rather than from the phenotype of its offspring. Although the methodology is now limited to genomes of the size of E. coli, in the future, Lerman will seek to display the DNA corresponding to a large deletion of a mouse chromosome, a rather more formidable task.

CONTRACTS

The Human Genetic Mutant Cell Repository (N01 GM 9-2101, Institute for Medical Research, Camden, New Jersey)

The NIGMS established on April 1, 1972, a respository of viable genetic mutant cells in culture for the purpose of stimulating research on human genetic diseases. Since its founding, the Cell Bank has resided at the Institute for Medical Researh (IMR) and, as a result of a five year renewal of the contract on January 1, 1979, will reside there through 1983.

The IMR is subjected to a site visit by the project officer and consultants at least twice per year, with the major review conducted in the spring. At the visit on April 9-10, 1979, the reviewers concluded that the Repository was functioning properly and was serving its constituency well.

In 1978 the Cell Repository established and stored 285 lines and shipped a total of 2,637 cell cultures, including 382 cultures to foreign countries. The Human Genetic Mutant Cell Repository published the 5th Edition of its catalogue in 1979. The catalogue listed 1700 lines from more than

200 biochemical diseases and 200 chromosomal anomalies. Sixteen new descriptions of cell cultures stored in the Repository were published or in press in 1978 in Cytogenetics and Cell Genetics.

The Cell Repository has several special Cell Collections, all of which are now considered complete:

a. Diabetic Cell Collection: This collection now contains 7 individual families consisting of 53 fibroblast and 47 lymphoblast cultures.

b. Huntington's Chorea Collection: This collection now contains both individual patients and 7 family groups, and consists of 32 fibroblast and 23 lymphoid lines.

c. Schizophrenia Cell Collection: This collection contains 27 fibroblast and 31 lymphoid lines and parts of 7 families.

d. Cystic Fibrosis Collection: This collection contains individual probands as well as members of 7 families and consists of 23 fibroblast and 11 lymphoid cell lines.

e. Xeroderma Pigmentosum Collection: This collection consists of 41 fibroblast and 78 lymphoid cell cultures, and all the complementation groups are represented. This group is considered essentially complete, but some refinement of family representation may occur.

In addition to these special cell line collections, 32 other disease collections are also now considered complete. The Institute and the Advisory Committee believe, however, that the Cell Repository is to be a functional bank and not a museum. Therefore, the needs of various research fields and use patterns of the disease collections will be continually monitored to guide our assessment of the adequacy of the collections.

The Human Genetic Mutant Cell Repository is undertaking several new initiatives to improve the functioning and completeness of the collection. The IMR is developing procedures for holding both skin and blood specimens in the frozen state in order to assess the documentation and quality of a potential cell line before the staff becomes committed to the large amount of work involved in establishing a cell line. In recognition of the rapidly growing importance of hybridomas, the IMR is soliciting for inclusion in the Bank the mouse and rat myeloma clones used in hybridoma production as well as a few key hybridoma lines. The needs and demands for hybridomas in human genetic research will be evaluated by the Advisory Commitee, and criteria and limits on this portion of the collection will be established. The IMR will also investigate the possible addition of a limited number of domestic animal and primate cell lines that have a potential for serving as models of their

human disease counterparts. In addition, the IMR is soliciting contributions for a Human Trisomy Collection. A set of cell lines, each clearly and stably trisomic for one of the 22 autosomes is the theoretical goal, although trisomies #1 and #17 have never been observed in nonneoplastic tissue.

The computerization of the Repository record files which began in November, 1977, is now virtually complete. The requirement of entering into computer storage all previous records as well as entering ongoing cell line data has strained the resouces of the IMR. However, the computer system is now in totally operational condition and is serving the data handling needs of the contract well. Experience has now revealed that the original computer capacity was insufficient and even that an initial expansion of that capacity will be insufficient to handle the ongoing data management requirements of the contract. IMR has been authorized to upgrade its computer capacity to a level that will be adequate for the remainder of the current contract.

A final point is appropriate in this annual report, the first after a five-year renewal of the contract. The advisory committee and the Institute believe that the Cell Repository is approaching a mature stage, and we expect to continue a policy of placing priority on improving the quality of the existing collection over expanding its size. In this mode, we will be able to provide the highest quality cell cultures and to maintain a catalogue of the most appropriate cell lines for current and future research needs.

Dedication

Having devoted ten years to the direction of the Pharmacology-Toxicology Program, Dr. Byron B. Clark retired from government service on January 12, 1979.

A Texan by birth, Dr. Clark received his B.A. degree from Baylor University, began his professional career as a research associate at the State University of Iowa Hospital, and received his M.S. and Ph.D. degrees from the S.U.I. His academic career began at Albany Medical College and continued at Tufts University School of Medicine where he served as chairman of the department of pharmacology. Prior to joining the National Institute of General Medical Sciences, Dr. Clark spent ten years as vice-president of the Mead Johnson Research Center in Evansville, Indiana.

Dr. Clark brought to the Pharmacology-Toxicology Program a continuing interest in expanding the frontiers of pharmacological research. He devoted special attention to stimulation of the development of the use of stable isotopes in studies of drug metabolism and in clinical pharmacology. Under his leadership the basic biorelated chemistry research supported by the NIGMS was integrated into the Pharmacology/Toxicology Program and increased areas of emphasis in molecular pharmacology and pediatric clinical pharmacology were initiated. He was especially interested in furthering interactions and common interests in pharmacological and toxicological issues with colleagues in other agencies of DHEW.

The grantees and staff of the Pharmacology-Toxicology Program join together in dedicating this year's annual report of our mutual efforts to Dr. Clark.

Research Overview

One of the most difficult annual tasks of the staff of the Pharmacology-Toxicology Program is to pick from many, the few research highlights to be included in the Institute annual report.

Several points may be noted. First, basic research into the mechanisms of toxic effects of drugs, particularly those related to pathways of metabolism, continues to be of special interest to the scientific community. Understanding the enzymatic and cellular basis for untoward effects of therapeutic drugs may lead to substitutes not as subject to toxic pathways, such as the use of N-acetylprocainamide for procainamide. Studies of enzymatic pathways related to production of toxicity may provide antidotes as in the use of 5-formyltetrahydrofolate in methanol poisoning. These studies may also lead to an understanding of basic biological mechanisms such as those related to lysosomal uptake of drugs by biological membranes.

The highlights provided by our grantees point to an imminent explosion of information related to stereochemical, stereosynthetic, and perhaps "stereopharmacological" effects. Work over the past few years on the therapeutic and metabolic differences between isomers of warfarin has now been extended to

a new methodology for probing ratios of different forms of metabolic enzymes in vivo. It has been known for a long time that different isomers of racemic drugs have different potencies and bind with different affinities to receptors. Grantees of the Pharmacology-Toxicology Program are exploring new analytical methods which can differentiate between isomers and assay for one in the presence of the other.

In synthesizing natural products by enzymic pathways, nature is always stereospecific. In complex series of laboratory reactions developed to prepare these same molecules and analogs of biological and pharmaceutical interest, chemists have not been able to be as selective. However, this year we have seen an increase of interest among our grantees in developing new methods of stereospecific syntheses of complex biological molecules.

A corollary of this is that as we learn to use more of the tricks which nature had previously reserved to herself, we are also learning more of the basic mechanisms underlying enzyme-mediated catalysis.

Finally, an examination of the highlights related to clinical pharmacology and therapeutic advances indicates that application of pharmacokinetic principles has become an integral part of these studies rather than an esoteric subdiscipline. Kinetic analyses of patient-derived data are providing clues to the presence of pharmacologically active metabolites of some therapeutic drugs as well as providing information about drug-disease interactions.

Research Manpower

Within the authority of the National Research Service Act, the Pharmacology-Toxicology Program supports research training in the pharmacological sciences and in clinical pharmacology. Institutional awards in pharmacological sciences at 54 institutions provided support for the graduate studies of 488 predoctoral fellows. These fellows, while receiving a solid common grounding in principles of pharmacological research, were located in schools of medicine, pharmacy, arts and sciences, and agriculture. Their doctoral degrees will be in disciplines including pharmacology, toxicology, biochemistry, physiology, medicinal chemistry, pharmaceutics, and genetics.

The 17 postdoctoral institutional programs in clinical pharmacology supported 57 trainees. These programs for physicians provide didactics as well as training in laboratory and clinical research. A meeting of the directors of these programs was held in May, 1979, in Bethesda to discuss the performance of the programs to date. It was concluded that several challenges face the programs in the future. They include the recruitment of sufficient numbers of suitable candidates with appropriate clinical backgrounds for the programs and giving the fellows a substantial didactic grounding in clinical pharmacology as well as solid training in laboratory and clinical research within a limited time frame.

Candidates for postdoctoral training in pharmacological sciences as well as clinical pharmacology may apply for individual postdoctoral fellowships. Currently 20 outstanding young scientists are supported by this mechanism in the Pharmacology-Toxicology Program.

The Pharmacology Research Associate Program of the National Institute of General Medical Sciences provides two years of postdoctoral research experience for twenty-two fellows in the intramural laboratories of the NIH, including NIMH. Over forty intramural scientists serve as potential preceptors with fellows participating in projects including studies of the mechanism of action of chemotherapeutic agents, the role of reactive intermediates in pharmacological and toxicological mechanisms, and regulation of neurotransmitter synthesis and action. During FY 1979, the training program in clinical pharmacology was more fully implemented with the initiation of a seminar series covering important aspects of clinical pharmacology.

Since July 1, 1977, the Pharmacology-Toxicology Program has accepted applications for research career development awards only from investigators whose career goals and research interests are in clinical pharmacology. It is the intent of the National Institute of General Medical Sciences that this program will foster the career development of a cadre of outstanding investigators who will integrate principles of basic research with problems of long-term clinical relevance. In FY 1979, no new awards were made. However, support continued for 15 individuals in diverse areas ranging from chemical and molecular studies to clinical pharmacology.

Organization and Management

The Pharmacology-Toxicology Program is divided into two major subsections, pharmacological sciences and biorelated chemistry. Dr. Carl Kuether has major responsibility for research activities in biorelated chemistry, while Dr. Anthony Zavadil administers programs in the pharmacology area, with a special interest in clinical pharmacology. Dr. Christine K. Carrico joined the program during FY 79 and is also responsible for research support in the pharmacological area with special interests in molecular and biochemical pharmacology. Each administrator also shares responsibility in overview of both institutional and individual fellowship training activities.

Dr. Folke Sjöqvist, Professor, Department of Clinical Pharmacology, Karolinska Institute, Huddinge Hospital, Huddinge, Sweden, who arrived in January 1979 to spend a sabbatical year, has served as Expert Consultant to the Director, NIGMS. Dr. Sjöqvist has been working with the staff of the Pharmacology-Toxicology Program devoting special attention to the new clinical pharmacology subprogram of the intramural Pharmacology Research Associate Program.

Following the retirement of Dr. Byron B. Clark, Dr. Sara A. Gardner was appointed Director of the Pharmacology-Toxicology Program in April, 1979.

RESEARCH HIGHLIGHTS

Mechanisms of Toxicity

"Studies on Methanol Poisoning"
GM 19420 (Tephly) University of Iowa

Methanol poisoning which occurs in man and does not occur in rats can be demonstrated in primates. Formate accumulation in the monkey is coincident with toxic features such as metabolic acidosis and visual impairment observed in the syndrome. Dr. Tephly and his associates have shown that when formate is given to monkeys, optic nerve toxicity occurs in a manner similar to that observed when methanol is administered to these animals. Why does formate accumulate in the monkey and produce a toxicity whereas it does not in the rat? Formate metabolism in the monkey has been shown to be lower than it is in the rat. A folate-dependent system is responsible for the metabolism of formate and functions in the oxidation of this compound to carbon dioxide. The level of folate in monkey livers is about half that seen in rats and the monkey metabolizes formate at about one-half the maximal rate observed in the rat. These observations have led to an experimental treatment for methanol poisoning in the monkey. Monkeys intoxicated with methanol were given repeated injections of 5-formyltetrahydrofolate (5-fTHF). When animals are either pretreated or treated simultaneously with 5-fTHF and methanol, there is a marked diminution of blood formate levels and no metabolic acidosis. In addition, there is an increase in the oxidation of formate to carbon dioxide. While the current optimal treatment for methanol poisoning is hemodialysis in combination with ethanol and sodium bicarbonate, these procedures are not readily available to the physician, nor are they universally effective, especially in children. For such situations, 5-fTHF therapy may prove to be important in the management of methanol poisoning in humans.

"Studies on the Pathogenesis of Drug-Induced Lipidosis"
GM 24979 (Hostetler) University of California, San Diego

In the early 1970's patients in Japan and Europe who had been taking a drug called Coralgil (4,4'-diethylaminoethoxyhexestrol) to lower blood cholesterol levels developed a disease characterized by lipid storage in body tissues. Several of these patients died as a result of this toxicity and the drug was removed from the market. In addition to Coralgil, it has recently become apparent that many other drugs with related chemical structures (but unrelated pharmacologic uses) may also cause lipid storage in body tissues. Using rats as a test system, Dr. Hostetler and his colleagues have been conducting experiments in an attempt to discover the molecular basis for this type of toxic drug reaction using Coralgil and chloroquine to study the nature of the lipid storage in rat liver. Rats were fed large doses of Coralgil and chloroquine by stomach tube for five days. At the end of this period the phospholipid and cholesterol content of the liver was found to be about 50 percent greater in the drug-treated animals.

In order to learn more about the possible mechanisms of the drug-induced lipid accumulation, subcellular particles were purified from the normal and drug-treated livers. Mitochondria, microsomes, nuclei, lysosomes and cell

supernatant fractions were isolated and their content of phospholipid and cholesterol was determined. Surprisingly, all of the phospholipid accumulation occurred only in the lysosomes. In addition, most of the excess cholesterol in the drug-treated liver was found in the lysosomes, the remainder being present mainly in the microsomal and supernatant fractions of the cell.

It was also found that chloroquine and Coralgil are concentrated to a remarkable degree in the liver lysosomes. The concentration of these drugs in lysosomes was 10 to 15 times the concentration of the respective drug in the liver homogenate. Extremely low levels of the drugs were found in the supernatant fraction of the liver indicating that they have a marked preference for biological membranes.

Dr. Hostetler's work thus far suggests strongly that the lipidosis-causing effect of these drugs must be related in some way to their tendency to concentrate in lysosomes. Since the lysosomes are also the primary site for the storage of excess lipid, it is likely that the lysosomal drugs interfere in some way with the degradation of phospholipids and perhaps with the degradation of cholesterol esters.

Clinical Pharmacology

"Pharmacokinetics of Salicylate in Children"
GM 19568 (Levy) State University of New York, Buffalo

Dr. Levy and his associates have been investigating the pharmacokinetics and metabolism of aspirin in children. Large-doses of aspirin are given for treatment of juvenile rheumatoid arthritis and dosage must be individualized and adjusted carefully to maintain plasma salicylate concentrations in the relatively narrow therapeutic range.

Monitoring the plasma concentration of some drugs may require the collection of several properly timed blood samples during a selected dosing interval, a procedure which can be uncomfortable, particularly to children. Dr. Levy's group has studied the time course of salicylate concentrations in plasma of 17 children, 4 to 17 years old, who received aspirin every 8 hours for the treatment of juvenile rheumatoid arthritis. The ratio of maximum to minimum steady state salicylate concentrations during the 8-hour dosing interval varied by less than 30 percent when the drug concentrations were at or above the usual minimal effective level. This information justifies the drawing of only a single blood sample during a selected dosing interval for monitoring salicylate therapy in children and indicates also that the timing of the blood sample during the dosing interval is not critical. Consequently, proper control of aspirin dosage is simplified, less demanding of nurses time, and more comfortable for the young patient.

"Kinetics of Drug-Drug and Drug-disease Interactions"
GM 22209 (Blaschke) Stanford University Medical School

Much of the work this past year has focused on the pharmacokinetics and pharmacodynamics of prazosin, a recently-marketed drug which produces vaso- and

veno-dilation. The mechanism by which the drug acts is uncertain and previous theories about its mechanism(s) have not been consistent with the observed effects in man. In view of its increasing popularity in treating hypertension, and in trials in congestive heart failure, studies were designed to examine its mechanism of action in humans, and to investigate the effect of diseases, particularly congestive heart failure, on its disposition.

Cardiovascular, catecholamine and neuroendocrine changes were studied following administration of prazosin to ten normal subjects. In response to a significant fall in standing blood pressure, heart rate (measured by continuous ECG monitoring) rose. Five of the ten subjects sustained their tachycardia on standing and developed at most mild symptoms. In the other five, tachycardia suddenly gave way to bradycardia. Isoprenaline infusion produced the same change in heart rate during the time of maximum prazosin action as when given alone.

In the supine position, when blood pressure was not significantly different from control, plasma norepinephrine concentration was elevated. In response to standing hypotension plasma norepinephrine was also elevated.

Four hours after taking prazosin 5 of the subjects stood for 30 minutes and plasma renin activity, cortisol and growth homone levels were measured. Plasma renin and cortisol were elevated in hypotension as was growth hormone. Prolactin did not rise significantly during hypotension induced by prazosin. These observations are not consistent with earlier reports that prazosin lowers blood pressure without producing a reflex increase in heart rate or renin release.

The pharmacokinetics of oral prazosin was studied in ten healthy normal subjects and in nine patients with congestive heart failure (CHF). After the initial dose, time to peak blood level was not significantly different between the normal subjects and patients with CHF. The area-under-the-curve for prazosin was significantly greater in patients with CHF than in normal subjects, and the elimination of prazosin was significantly slower in CHF patients. These data suggest that in patients with CHF the elimination of prazosin is substantially slower than in normals. Therefore, higher steady-state prazosin concentrations can be expected in CHF patients compared with normal subjects given the same dose.

These findings suggest that the mechanism of action of this drug may be different from that proposed from studies in vitro and in experimental animals, and that, at least acutely, the drug has effects similar to those of other vasodilators such as nitroprusside. The "first dose phenomenon" with prazosin is probably related to inadequate venous return to the heart and bradycardia and knowledge of this mechanism should allow it to be avoided in virtually all patients. Finally, it appears that the hepatic elimination of prazosin is flow-dependent, and it therefore must be used with caution in patients with altered hepatic blood flow (as in CHF or portosystemic shunting).

New Approaches to Therapy

"Design of Chemotherapeutic Agents"
GM 16492 (Talalay) Johns Hopkins University

Last year, Drs. Talalay and Bueding reported two novel and potentially important principles for improving the safety of a variety of chemotherapeutic agents and reducing the toxicity of certain chemicals. These principles are: (1) prevention of the metabolic activation of the compounds in question by microorganisms of the intestinal flora, and (2) administration of dietary antioxidants which enhance the levels of certain enzymes that inactivate potent mutagenic and carcinogenic metabolites. Continued attention has been directed toward the mechanisms, scope, and applications of these protective measures.

Administration to various species of poorly absorbed antibiotics, such as erythromycin, prevents the conversion of the potent antischistosomal isothiocyanate CGP 4540 (amoscanate) to a mutagenic product(s). Although this isothiocyanate is in general nontoxic, doses exceeding the curative dose by a factor of 25 can produce lesions of the endothelial lining of the bile ducts and gallbladder. Coadministration of erythromycin (or other poorly absorbed antibiotics, such as paramomycin), markedly reduced the biliary tract toxicity. It appears that amoscanate is converted by one or several components of the enteric bacterial flora not only to a mutagen, but also to a product(s) that has specific biliary toxicity. These same antibiotics do not affect the antischistosomal activity of the drug. Although the dose required to induce biliary tract lesions is much higher than the curative schistosomicidal dose of amoscanate, the antibiotics would provide an additional protective factor against toxic complications in particularly sensitive individuals. Thus, the possibility of inhibiting toxic effects, while maintaining full chemotherapeutic activity by means of enteric antibacterial agents, suggests new approaches of more general applicability to drug therapy.

In further studies of this problem, an intestinal microorganism capable of converting amoscanate to a mutagen has been identified. The bacterium, isolated from the intestinal tract of mice, was found to produce mutagenic activation of amoscanate when introduced either into animals pretreated with antibacterial agents or into germ-free animals. This organism has been identified by Dr. Facklam at the Center for Disease Control, Atlanta, Georgia, as Streptococcus equinus. Several other species of streptococci (S. bovis, S. mutans, S. intermedius, and S. uberis) have been found to convert amoscanate to mutagens. Some of these species are known to be constituents of the intestinal flora of man. It is hoped that this information will guide efforts to reduce the mutagenicity of other drugs.

Drug Metabolism

"Product Ratio and Microsomal Multiplicity"
GM 25136 (Trager) University of Washington

Dr. Trager's laboratory has been investigating the effects of inducing agents on the metabolism of two structurally similar anticoagulants, warfarin and phenprocoumon. Treatment of rats with 3-methylcholanthrene, a carcinogenic benzpyrene-like inducing agent, lead to the stereoselective formation of two specific metabolites of warfarin. These are (R)-6 and 8-hydroxywarfarin. If phenprocoumon is incubated with the liver microsomal fraction from these same animals, two specific metabolites are stereoselectively formed as well; however, these are (S)-6- and 8-hydroxyphenprocoumon. The intriguing aspect of this observation is that although the two compounds are subjected to similar metabolic transformations from the same enzyme complex, a complete reversal in stereoselectivity results. If the experiments are repeated using β-naptho-flavone, another benzpyrene-like agent, similar results are obtained. Conversely, if the animals are treated with inducing agents that are different from benzpyrene (e.g., phenobarbital), the reversal in stereoselectivity is not observed. Dr. Trager thinks that the metabolic patterns of warfarin and phenprocoumon may provide a highly sensitive and specific test for the presence of P-450 enzymes associated with carcinogenesis and that such an assay could be useful in screening the potential hazards of new pesticides and herbicides and/or provide a means of quality control for presently utilized agents of this nature. In principle, it should be capable of detecting these critical enzymes even in the presence of complicated mixtures of enzymes that might result from agents which cause mixed induction.

Molecular Pharmacology

"Conformationally Defined Adrenergic Agents"
GM 22988 (Grunewald) University of Kansas

Dr. Grunewald has been studying the effect of the three-dimensional conformation of drug molecules on their mechanism of action. In collaboration with Dr. Charles O. Rutledge, this group has observed that the aromatic ring is not a requirement for high pharmacological activity of many drugs that contain such an aromatic ring as part of their chemical structure. Imipramine and desiprimine are aromatic drugs, and it has been assumed that this ring is an essential portion of the molecule for high pharmacological activity. One of the major effects of such drugs is to block the reuptake of neurotransmitters into presynaptic nerve terminals. To explore the importance of the aromatic ring in such drugs, analogs of imipramine and desipramine were prepared by chemically-selective methods in which one or both of the benzene rings were reduced to fully saturated rings. The stereochemistry of the ring junctions was established by X-ray crystallographic analysis of the compounds so the actual three-dimensional shape was known. If an aromatic ring were required for some specific interaction involving the pi-electrons of that ring with an electron-deficient site on the neurotransmitter reuptake site, then one would expect the partially or fully saturated analogs to be less active or quite inactive. On the contrary, the partially reduced (one ring) or fully reduced

(both rings) compounds were as potent at inhibition of uptake of dopamine or serotonin into chopped rat cortex tissue as the fully aromatic parent, thus suggesting that a hydrophobic interaction of the rings is all that is important and no specific requirement of the aromatic ring is involved. However, at the site for reuptake of norepinephrine, the saturation of one or both aromatic rings resulted in a 150- or 250-fold decrease in potency, respectively, on uptake of norepinephrine. This suggests that either a specific pi-electronic interaction is important for the binding of drugs such as imipramine and desipramine at the norepinephrine reuptake site, or that the steric changes in shape of the saturated molecules compared to the parent aromatic drugs are of considerable importance at the norepinephrine site but not at the site for reuptake of dopamine or serotonin. This work should permit the design of more selective drugs to modulate this important mechanism of controlling activity of the nervous system.

"Characteristics of PGE-Sensitive Adenylate cyclase"
GM 25163 (Perkins) University of North Carolina

Dr. Perkins and his associates have developed a new method for the purification of mammalian cell plasma membranes and have applied it to the purification of hormone-sensitive adenylate cyclase. Human astrocytoma cells were treated with the lectin concanavalin A, washed, homogenized and the homogenate centrifuged over a sucrose density gradient. This procedure caused the plasma membrane to sediment as a narrow band at an uncharacteristically high density. When this plasma membrane fraction was examined with the electron-microscope, it was found to be characterized by large extended sheets. When the bound concanavalin A was removed by adding alpha-methylmannoside to such preparations, the plasma membrane was observed to sediment at a much lower density of sucrose, similar to the sedimentation characteristics of plasma membrane from cells not treated with the lectin. The preparation obtained on the second gradient is enriched 15-25 fold in various plasma membrane marker enzyme activities. The procedure is simple and can be accomplished in about three hours. Adenylate cyclase has been purified up to 26-fold with about 20 percent recovery by this method.

During the development of this procedure, it was observed that not all of the β-adrenergic receptors of the human astrocytoma cells migrated with the plasma membrane on the sucrose gradient. This light density fraction of β-receptors exhibited the same pharmacological properties as β-receptors in the plasma membrane in terms of ligand binding, but the fraction contained little or no adenylate cyclase activity. The proportion of β-receptors found in the light density fraction was correlated with the rate of β-receptor synthesis at two stages of cell growth. The tentative conclusion from this is that the light fraction of β-receptors may represent an intracellular vesicular form of the receptor which could be a precursor of the plasma membrane form of the β-adrenergic receptor.

New Methods

"Alteration in Drug Metabolism by Certain Carbamates"
GM 16503 (Fujimoto) Medical College of Wisconsin

In contrast to the numerous methods for studying the renal elimination of drugs and xenobiotics by the kidney, a paucity of techniques applicable to the study of hepatic function has prevented a more thorough understanding of mechanisms of biliary excretion. To fill this gap, Dr. Fujimoto's laboratory has recently developed a technique called segmented retrograde intrabiliary injection (SRII) which is applied to the rat. This technique has permitted the study of certain characteristics of biliary tree function.

The principle of the SRII is to deliver an initial "segment" of solution containing a radioactive marker compound into the biliary tree and wash this marker in with saline solution in excess of the biliary tree capacity. Bile is immediately re-collected and the quantitative distribution of the radioactive compound is measured. By this technique, it can be shown that the biliary tree epithelium (canalicular membrane) acts as a molecular sieve for filtration as determined by using solutes of varying molecular size. Furthermore, it was shown that D-glucose was taken up into the liver to a greater extent than expected from its molecular size. The fraction which is taken up was shown to involve a carrier-mediated uptake system since it could be competitively blocked by intravenous administration of phlorizin, a toxic glycoside which blocks glucose uptake. Phlorizin had no effect on uptake of mannitol, 3-O-methylglucose or sucrose.

The glucose uptake system was also competitively blocked by administration of unlabeled glucose or methyl-α-D-glucopyranoside. Therefore, there exists a transport system in the biliary tree for selectively reabsorbing glucose from bile. This mechanism may be important in conserving D-glucose in the body. The SRII technique can now be used to study other systems for reabsorption of drugs, nutrients, and xenobiotics from the biliary tree.

"Mass Spectrometry of Non-Volatile Organic Compounds"
GM 19749 (Cook) University of Illinois

Although mass spectrometry is an exceptionally powerful technique for chemical identification, its application to compounds of biomedical interest has been severely limited by the need to vaporize and ionize sample molecules. In conventional analyses, compounds are vaporized by heating and ionized by bombardment with high energy electrons. Many organic compounds undergo significant degradation during these early stages of analysis.

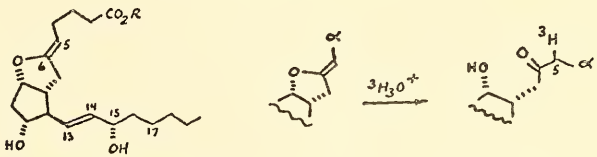
Electrohydrodynamic (E.H.) ionization offers an alternative and much gentler approach to these preliminary analysis steps. Samples are simply dissolved in an appropriate solvent in which sample ions are formed either by simple dissociation (in the case of salt-like samples) or by chemical attachment of protons, other cations, or anions to intact sample molecules. Interaction with a strong electric field subsequently promotes the emission of these ions from the solution into the mass spectrometer. Glycerol is usually the solvent of choice due to its low volatility and good solvation properties.

This approach has allowed mass spectral analysis with little or no degradation of a variety of biomedical and other compounds which could not be sampled without fragmentation by any other ionization technique. In fact, E.H. ionization is more susceptible to molecular "growth" than fragmentation; ions undergoing analysis frequently retain one or more solvent molecules as they enter and pass through the mass spectrometer. It is usually possible to determine the number of attached solvent molecules for ions passing through the spectrometer undisturbed (e.g., by comparing data from solutions prepared with slightly different solvents). Problems arise, however, when weakly bound solvent molecules evaporate from cluster ions within the spectrometer. Because of this evaporation process, ions detected are not identical to ions emitted; they can be smaller (lighter) due to loss of solvent molecules. However, the ions emitted should most closely reflect the composition of the sample solution. Thus, to gain extremely useful quantitative information about sample composition, the relation between detected and emitted ions must be established. By careful selection of experimental parameters, Dr. Cook has been able to distinguish among four kinds of detected ions: (1) those initially emitted from solution and not undergoing any evaporation, (2) products of solvent evaporation taking place between the ion source and the first mass focusing element of the AEI MS902 mass spectrometer, (3) products of solvent evaporation taking place between the first and second focusing elements of the MS902, and (4) products of both evaporations described in (2) and (3) above. As an understanding of the dynamics of the evaporation process develops, electrohydrodynamic ionization mass spectrometry may prove a valuable tool for such diverse analyses as peptide sequencing and quantitation of biomedical compounds in solution.

Macromolecular Biosynthesis

"The Molecular Basis of Prostaglandin Biochemistry"
GM 20848 (Andersen) University of Washington

Dr. Andersen and his co-workers have extended their studies of the stereochemical features requisite for biorecognition and differentiation of prostaglandins to the newest member of this class of potent regulatory agents, prostacyclin (I). Prostacyclin is an unstable, but highly potent, agent implicated in the control of blood pressure and the dissolution of platelet thrombi. A number of chemically synthesized analogs display increased selectivity for these two distinct actions. For example, formation of the 15-methyl ether completely eliminates the platelet antiaggregatory activity but the analog retains the vasodilatory action. Removal of the



I (R=H), prostacyclin
II R=CH3

III

13,14-olefinic linkage has the opposite effect, the analog has less than 5 percent activity on blood pressure but is one-half as active as natural prostacyclin in protecting platelets. The last mentioned analog is of particular interest since it can be anticipated to be a natural metabolite of prostacyclin with an increased *in vivo* half-life in circulation.

The chemical instability of prostacyclin (PGI_2) is also a major problem in developing assay methods for PGI_2. PGI_2 hydrolyzes to 6-keto-PGF_1 (part Structure III) with a half-life of 6 min at pH 7.4 in aqueous buffers. As a result most assay methods are indirect, based on determination of III by chemical or radioimmunoassay methods. Increasing evidence points to the conclusion that only a small portion of PGI_2 goes through III in its *in vivo* detoxification. Assay methods based on III are thus suspect.

Recently Dr. Andersen's research group realized that the hydrolysis reaction which has hampered previous effort to directly quantitate PGI_2 could be used to advantage by incorporation of tritium (^3H) into the molecule. They have determined that hydrolysis in ^3H_2O leads to incorporation of up to 60 percent of the theoretical quantity of ^3H at C-5 in III. In contrast, 6-keto-PGF_1 incorporates ^3H (via enolization) only slowly under these conditions giving specific activities less than 10 percent of those obtained from intact PGI_2. This assay thus provides a way to distinguish intact prostaglandins from their hydrolysis products. The current detection limit is 30 ng of PGI_2. Further study could provide a clinically useful assay for this important humoral agent.

Prostacyclin ester II has been shown to have an extended half-life (20 times that of I in aqueous ethanol). *In vivo* studies in the baboon reveal that this increased chemical stability imparts a longer period of action implying an increased half-life in circulation as well.

Natural Products

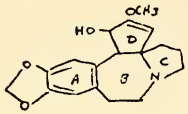
"Biosynthesis of the Antileukemic *Cephalotaxus* Alkaloids"
GM 26569 (Parry) Rice University

Cephalotaxus is a genus of conifers that occurs in the Far East. Some years ago, the plants in this genus were shown to contain an unusual group of alkaloids, the major one of which is cephalotaxine (1). It is accompanied in *Cephalotaxus* by minor amounts of several ester alkaloids including deoxyharringtonine (2), isoharringtonine (3), harringtonine (4), and homoharringtonine (5). The alkaloids 2-5 exhibit anticancer activity, and, in the People's Republic of China, harringtonine and homoharringtonine have been reported to be effective in the treatment of human cancers.

Over the past several years, Dr. Parry and his colleagues have been investigating the biochemical pathways that *Cephalotaxus* plants utilize for the manufacture of the anti-cancer alkaloids. Initially, it was shown that the parent alkaloid cephalotaxine is derived from one molecule each of the aromatic amino acids phenylalanine and tyrosine. The mechanism of formation of cephalotaxine from these amino acids appears to bear a resemblance to

the formation of the antimitotic alkaloid colchicine in Colchicum plants. More recently, it has been demonstrated that the conversion of phenylalanine into cephalotaxine proceeds with loss of one of the meta carbon atoms from the aromatic ring of this amino acid. The loss of the meta carbon leads to the creation of the five-membered D ring of cephalotaxine.

Another aspect of recent work on the biosynthesis of cephalotaxine concerns the elucidation of the metabolic relationships between cephalotaxine and three closely related alkaloids, cephalotaxinone (6), desmethylcephalotaxinone (7), and desmethylcephalotaxine (8). Desmethylcephalotaxine and desmethylcephalotaxinone are derived by irreversible demethylation of cephalotaxine and cephalotaxinone, respectively. The metabolic relationships between the four alkaloids 1, 6, 7, and 8, therefore, resemble those that have been shown to exist between the three opium alkaloids, thebaine, codeine, and morphine.



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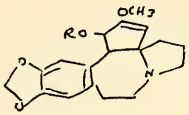
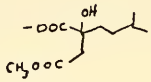
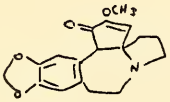


2, R= 



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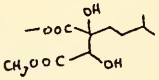
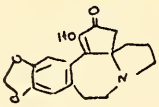
3, R= 



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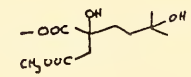
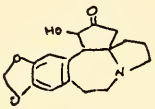
4, R= 



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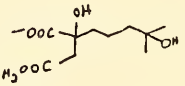
5, R= 

Precursor incorporation experiments with deoxyharringtonine (2) demonstrated that the diacid attached to cephalotaxine in deoxyharringtonine is formed in vivo from the amino acid leucine. The route utilized by Cephalotaxus to synthesize the deoxyharringtonine diacid appears to resemble closely the pathway used in microorganisms for the biosynthesis of leucine from the amino acid, valine.

"ACTH and Related Peptides"
GM 02907 (Li) University of California, San Francisco

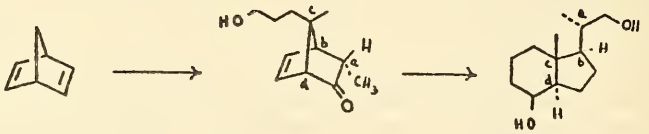
During the course of isolation of β-lipotropin and β-endorphin, Dr. Li and his colleagues obtained a new peptide (4.5 mg) from frozen whole human pituitary glands. Structural studies of the peptide gave an amino acid sequence identical to α_h-ACTH-(7-38). The solid-phase synthesis of the peptide was achieved by procedures similar to those used for the synthesis of human β-endorphin. Purification of crude synthetic material was effected by chromatography on CM-cellulose and partition chromatography on Sephadex G-25 to give α_h-ACTH-(7-38) in 18 percent overall yield.

The melanocyte-stimulating activity of the peptide, as assayed by the frog skin procedure, amounted to 30 percent the potency of the native ACTH. It is devoid of corticosteroidogenic activity. In isolated rat adrenal cells, the peptide inhibits the steroidogenic effect of ACTH. It was also found that it inhibits ACTH action on cyclic AMP production in vitro and in vivo. Thus, α_h,ACTH-(7-38) was named corticotropin-inhibiting peptide (CIP). This would seem to be the only known peptide from a natural source capable of antagonizing the adrenal-stimulating activity of ACTH. It is not inconceivable that its production might be responsible for the development of adrenal insufficiency.

Synthetic Chemistry

"Macrolides, Steroids, Prostanoids, Etc. -- Synthetic Design"
GM 13598 (Trost) University of Wisconsin, Madison

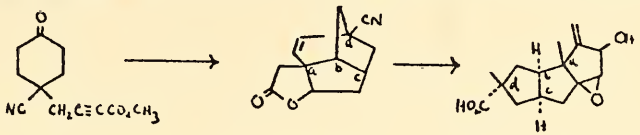
Dr. Trost is interested in the creation of new members of classes of structures closely related to natural products but not readily available from them. Vitamin D constitutes one such class. The metabolites of this vitamin, which are present in minute quantities, have been found to be the active forms of the vitamin. Dr. Trost and his co-workers have developed a fully controlled, facile synthesis of a key fragment, which has been termed the Inhoffen-Lythgoe diol 1, utilizing such methodology. The key point is the utilization of a bridged polycyclic structure to control stereochemistry. The important centers in 1 are lettered and correspond to carbons 20, 17,



13, and 14 respectively in the vitamin D metabolites. These letters are also translated to the corresponding carbon atoms in the key bicyclic compound 2. The places of substitution in the active vitamin are in the side chain and ring A. These two units can easily be added to 1. The fact that the

precursor of 2 is ultimately norbornadiene 3, an achiral substance, allows a chiral route to evolve.

This strategic principle for controlling stereochemistry has also been successfully applied to the synthesis of hirsutic acid 4. This compound represents the first member of a class of novel natural products that possess antibiotic and antitumor activity. The creation of the key bicycle 5 involved



6 5 4

a new type of carbon-carbon bond forming reaction in the key step. The evolvement of this reaction, which should be of general applicability, was crucial to the efficiency of this approach. It involved the equivalent of inverting the reactivity of a carbonyl group to effect cyclization by treating an aldehyde with thiazolium salt. This reaction mimics the behavior of vitamin B_1 in biological systems and may be considered a biomimetic approach.

The source of the bicycle 5 is an achiral compound 6. The cyclization of 6 when performed with quinine generated, after several additional steps, 5 which was optically active to the extent of being 65 percent of one enantiomeric configuration. Thus, this concept not only provides a strategy which allows full stereochemical control at all centers but also has the possibility of a chiral synthesis.

PHYSIOLOGY AND BIOMEDICAL ENGINEERING PROGRAM

The past year has been one of administrative and programmatic consolidation of the Physiology and Biomedical Engineering (PBME) Program. It began, regretfully, with the departure of the Acting Program Director, Dr. Peter Abbrecht, who had helped the Institute to bring the Clinical and Physiological Sciences and Biomedical Engineering Programs together. The PBME Program has acquired one new professional staff member, Dr. Bert Shapiro, whose main responsibilities, however, remain in the Cellular and Molecular Basis of Disease Program. Because of his broad knowledge and interest in physiology, particularly neurobiology, he took on the task of administering the institutional National Research Service Awards in Systems and Integrative Biology in December 1978. In all other respects the professional staff of the PBME Program has been stable.

The classification of program activities into sections and subprograms was completed, and specific Program Administrators assigned responsibility for each. While there is little overlap between the various subprograms, the PBME Program is fortunate in having extensive ties with the other Institute programs through the sharing of professional staff. Thus three members of PBME have "joint appointments" in other programs.

The PBME Program encompasses grant support for quite diverse activities, such as instrument and method development; the application of engineering sciences to biological problems; biomedical research focused on clinical problems encountered in anesthesiology and after severe trauma; certain fundamental problems in neurobehavioral science. Throughout this wide range runs a common thread, which is an emphasis on biological problems. Thus instrument development is expected to concentrate on new or improved instruments that are needed critically for the pursuit of biological research. Clinical research is expected to lead to the discovery of the biological mechanisms deranged after trauma, for instance.

As is apparent from the above, the PBME Program is primarily concerned with finding solutions to biological research problems. In earlier years, the development and encouragement of discrete disciplines was viewed as a necessary, concomitant goal, but as the emerging disciplines have become established, and with the increasing competition for grants, the relative need and priority for this has decreased. However, the availability of suitable instruments and well-trained investigators is still a limiting factor, and the PBME Program provides support in both areas, ultimately benefiting other programs in the Institute and in other institutes.

INSTRUMENTATION

In order to give investigators greater access to new or renovated research equipment of intermediate cost, the Institute announced in January 1979 a one-time-only offering of grant support for shared research equipment. Although primarily intended for NIGMS grantees, it was also hoped that the grants would allow new instrumental techniques to become disseminated widely among potential users. A major feature was to limit the RFA, which was published in the NIH

Guide for Grants and Contracts on January 19, 1979, to analytical instruments in the cost range of $40,000 - $150,000, with maintenance and technical support during two subsequent years allowable up to $15,000 per year. Upon termination of the grants, the Institute plans an evaluation of the instruments' utilization.

The single deadline was March 15, 1979, and the merit reviews were performed by DRG during the ensuing four months. Council review is scheduled for August 1979, and funding of the most meritorious applications, for September 1979. The administration and evaluation of the program is the responsibility of the PBME Program, and Dr. Cassman, specifically. The following numbers of applications were received: radiology, 4; cell sorters, 14; x-ray crystallography, 16; protein sequencing, 24; electron microscopy, 34; mass spectroscopy, 35; spectroscopy (other than magnetic resonance), 36; and NMR/EPR, 49. It is anticipated that about $5 million will be awarded for this program in FY 1979.

RESEARCH TRAINING

Under the National Research Service Act of 1974, the PBME Program continues to support three institutional fellowship programs. One is a predoctoral program in Systems and Integrative Biology, the others, postdoctoral programs in Anesthesiology and in Trauma/Burn Research. The Program has made special efforts to communicate its intent in the area of training for research in Systems and Integrative Biology. In the past, this area was defined somewhat loosely and incorporated training in areas supported by the Institute under the old PHS authority that strictly could not be considered Systems and Integrative Biology. As the initial five-year project periods are coming to an end, so is this transition to the program announced under the NRS Act. An unresolved problem remains with regard to the "border" between systems and integrative biology and neurobiology, which would a priori be considered part of the mission of the National Institute of Neurological and Communicative Disorders and Stroke. The latter Institute is concentrating its interest on postdoctoral support, while NIGMS has a broad emphasis in predoctoral training.

The PBME postdoctoral training programs have come under a general review during the year, with a conclusion that post-M.D. research training has not been adequate. The trainees have often not been given the minimum of two years of research training in basic science departments, as stated in the program announcement. Related problems have been observed in some of the other postdoctoral training programs of the Institute, and they have been the subject of extensive discussions with peer review groups, the NAGMS Council, and various other consultants. These discussions are part of a continuing effort to strengthen the quality of post-M.D. research training, even if it makes recruitment of candidates more difficult. We feel that in the long run the numbers of individuals trained is less important than the quality of the research training they receive.

CENTER GRANTS

The centers programs are going through a difficult period. As existing programs come up for competitive renewal, some of them fail to meet the scientific merit standards of peer review groups and are discontinued. Others show

remarkable vigor and scientific depth and have been continued, either as program projects or center grants, depending on their scope. A few new applications are being received, but most of the strong applicants have already joined the program. The anesthesiology centers program is having serious problems. Only two grants now remain active after recent peer reviews of all five. The number of research project grants (R01) in anesthesiology also is small. In view of this, the PBME Program has decided to place greater emphasis on the support of research training, so that future applicants will have had adequate scientific backgrounds to prepare proposals of high merit.

NEW INVESTIGATOR AWARD

In the same vein, the PBME Program supports new investigator awards (R23) for first-time applicants for research support in selected fields. These provide a mechanism of support for the transition period between research training and full-fledged, independent investigation. This mechanism is available for research on trauma and burns, and in anesthesiology. There are currently six active grants in the former area and 13 in the latter.

RESEARCH HIGHLIGHTS

1. "Biophysical Basis for Thresholds in Bio-Ultrasound"
GM 08209-15 (Nyborg) University of Vermont

Ultrasound is being used in a variety of clinical areas, which include diagnosis (particularly for in utero fetal monitoring during pregnancy and delivery) and therapy (deep tissue heating). Comparable frequencies are used for both types of applications, although the actual power levels (intensities) used for diagnostic purposes are a thousand times less than those used for therapy (1-4 watts). The procedures are considered to be "safe" and the method of choice over X-ray and radioisotopes for the routine examination of patients in gynecological departments in major hospitals and medical centers throughout this country. It has been estimated that nearly 40 percent of all obstetric patients in this country are subjected to ultrasonographic methods of examination.

Dr. Wesley L. Nyborg and his colleagues at the University of Vermont, with the support of the NIGMS, have worked on the biological effects of ultrasound for the past 15 years (GM 08209-15). Some years ago this group demonstrated that the presence of stable, minute air bubbles in an ultrasonic beam caused amplification effects to occur at energy levels far below those required to generate bubbles de novo. Air bubbles can be maintained within the pores of polycarbonate membranes when they are immersed in appropriately aerated solutions. These stabilized micro air bubbles respond to the pressure variations induced in an ultrasonic field with volume pulsations, thus themselves becoming small secondary sources of ultrasonic radiation.

The exposure of human platelet-rich-plasma, in contact with this type of membrane, to 32 mW/cm^2, 64 mW/cm^2 and 125 mW/cm^2 of continuous ultrasonic radiation for 10 minutes resulted in the agglutination of the cells at the two higher levels of energy. Furthermore, this group found that

adenosinetriphosphate was leached from the platelets by the same energy levels of ultrasound as used for diagnostic purposes.

These in vitro data are the first indication that very subtle events could occur in the mammalian cell during ultrasound irradiation.

There is no indication, at this time, that human cells in situ are subjected to the special environment of the micro bubbles described in Dr. Nyborg's experiments. However, it is known that certain plant cells with large numbers of gas-filled vacuoles are very sensitive to ultrasonic irradiation. Nevertheless work continues in this and other laboratories to better define the limits of "safe" ultrasonography as more is learned of microenvironments of irradiated systems and the conditions which cause irreversible biological changes.

2. "Ion Microscopy in Biology and Medicine"
GM 24314-03 (Morrison) Cornell University

The transport and compartmentalization of inorganic ions are important factors in a variety of biological phenomena. In particular, inorganic ions such as Ca^{++} and K^{+} are increasingly recognized as having crucial roles in the regulation of important cell functions. Accordingly, there is a great interest in techniques for localizing ions in cells and tissues. Developments in two major areas are necessary on the technical side, in order to investigate these problems. First, instruments capable of detecting these and other important ions within useful resolution limits must be developed. Second, techniques are needed for the preparation of cells or tissues without disturbing the original ionic distribution. In this report, Dr. George H. Morrison of Cornell University demonstrates the use of the Cameca IMS 300 ion microscope in a plant tissue where ion distributions appear to be important.

Although noteworthy findings about elemental composition have resulted from the use of electron microprobe X-ray analyses, point-by-point mapping of an element's distribution can be tedious. The Cameca IMS 300 ion microscope is designed with unique ion optics that allow direct microscopic imaging and thereby a one-to-one correspondence between the lateral distribution of the ions on the sample surface and that of the final mass-resolved image. The resultant image has a spatial resolution of 1 μm and a field diameter of about 250 μm. The images in this study were produced by bombarding the sample with an O_2^{+} primary beam and focusing and accelerating the secondary ions through the entrance slit of a double-pass magnetic sector for momentum filtering. An electrostatic mirror positioned after the first pass of the magnet allows the ion beam to be energy filtered. Thus a mass spectral analysis of the ions present within the areas of interest may be obtained. The secondary ion image, which is subsequently converted to an electron image, may be projected onto a fluorescent screen or otherwise recorded and counted.

The leaves of Mimosa pudica L., the sensitive plant, rapidly respond (< 1 second) to touch and other stimuli with movement. The mechanisms for these may well be analogous to those seen in animals, but appear to be basically

simpler and therefore easier to study. The leaf movements result from changes in the curvatures of specialized motor organs, the pulvini. The pulvinar curvatures result from differences in turgor of cells in opposing regions of the pulvinus. This requires accumulation or loss of water by the motor cells, and it is likely that this water movement is an osmotic consequence of redistribution of potassium ions within the pulvinus. The response and recovery of pulvini are also associated with relocation of calcium ions in the motor tissue, although the function of this Ca^{++} movement is not known.

The main pulvinus of Mimosa is a cylinder consisting of a central vein surrounded by a thick cortex of motor cells. This cortical region has two distinct concentric histological zones. The outer zone is characerized by cells with large tannin bodies. Most of the cells in the inner zone (near the vein) lack tannin bodies, but have central vacuoles generally containing a non-liquid material.

Using unstimulated pulvini, ion micrographs were taken of the outer zone which indicated that the tannin bodies and cell walls were relatively rich in both potassium and calcium. However, in the inner zone, K^{+} and Ca^{++} were segregated, with calcium abundant in the cell walls but scarce within the protoplasts, and potassium absent from the walls but plentiful in the central vacuole. Since the cells of the two zones of the main pulvinus differ both in structure and in the distribution of key ions, Dr. Morrison and his collaborators suspect that these two cell types differ in function. The observation that the tannin body has a high calcium content corroborates the hypothesis of other workers that the tannin body functions as a Ca^{++} reservoir. Since they also observed that the tannin body is a K^{+} rich site, it is probable that the role of this structure in rapid leaf movements is complex.

Although much further work is needed to elucidate mechanisms whereby these ion distributions effect movement in the sensitive plant, exact determination of ion localization during different functional states is a keystone to this effort.

3. "Fetal Cells in the Blood of Pregnant Women: Detection and Enrichment by FACS"
GM 17367 (Herzenberg) Stanford University

Dr. Leonard A. Herzenberg and his colleagues at Stanford University recently reported that they had been able to isolate and study fetal cells from maternal peripheral blood. The technique, which only requires 20 ml. of the mother's blood, is likely to become an important adjunct to amniocentesis for prenatal diagnosis of genetic anomalies.

Fetal cells among maternal peripheral blood lymphocytes (1/50,000) were stained by indirect immunofluorescence by using first a rabbit antiserum directed against paternal HLA cell-surface antigens absent in the mother, followed by a flourescein-conjugated goat anti-rabbit immunoglobulin. The blood lymphocytes were then sorted on a fluorescence-activated cell sorter,

and a fluorescein-positive enriched population of cells obtained, that proved to be Y-chromatin-positive on histochemical examination.

This new technique represents an advance over amniocentesis in that it carries less risk to the mother and child. However, its wide applicability remains to be determined, and will depend on the availability of usable specific antigenic differences between mother and child.

CONTRACTS

1. "Data Base for Gaseous Ion Thermochemistry"
(Interagency Agreement 3-Y01 GM 90009)
National Bureau of Standards ($26,400)

The contract to the National Bureau of Standards, with Dr. Henry M. Rosenstock as Principal Investigator, is for the compilation and evaluation of ionization and appearance potentials of positive ions. Such values are of importance to investigators working in the fields of mass spectroscopy, photoelectron spectroscopy, and certain areas of optical spectroscopy. Dr. Rosenstock and his colleagues not only compile values which are diffusely distributed through the literature, but also evaluate the measurements with regard to their probable reliability. Other contributions under this contract include reviews of the experimental techniques used to provide the listed data, and theoretical interpretations of fragmentation mechanisms.

2. Demonstration of Laboratory Sample Identification System
(Contract N01 GM 6-2131)
Georgetown University Medical School (No-cost extension, FY 1979)

Under this contract, the Georgetown University Clinical Laboratory has developed a positive patient-sample identification system of considerable complexity but with impressive effectiveness.

During the current no-cost extension period, each analytical instrument in the clinical laboratory will be tied into the identification system and checked out against a rigid set of criteria previously developed for the project. Since many of the analytical instruments employ analog signals as indicators for chemical reactions, and these signals are frequently of different orders of magnitude, the collation and interpretation of perhaps 30 or 40 individual signals feeding into a single central processing unit represents a programming effort of considerable magnitude. The remaining contract time during this year will be spent in integrating this laboratory system into an already functioning hospital information system. When complete, the laboratory system will be capable of receiving and sending input and output signals to and from the registrar's office, admitting service, pharmacy, and other services.

In addition, several tests have been made of the applicability of the newly developed hi-density bar code for the clinical laboratory.

SCOPE

The Minority Access to Research Careers (MARC) Program is institutionally based and is designed to assist minority colleges and universities in the training of greater numbers of scientists and teachers for careers in the biomedical sciences. It was designated as a formal program of the National Institute of General Medical Sciences in December 1975. Applications assigned to this program are reviewed by the Minority Access to Research Careers Review Committee, which was chartered specifically for the review of such applications.

OBJECTIVES

The primary objectives of the program are to assist minority institutions in the training of greater numbers of scientists in the biomedical disciplines and to help strengthen the research and teaching capabilities of these institutions. The support mechanisms used to accomplish this mission are: The MARC Faculty Fellowship, the MARC Visiting Scientist Award and the MARC Honors Undergraduate Research Training Program.

RESEARCH TRAINING

The MARC Faculty Fellowship program provides opportunities for advanced research training for selected faculty members of four-year colleges, universities and health professional schools in which student enrollments are drawn substantially from ethnic minority groups. These institutions may nominate faculty members to apply for MARC Fellowships for support for a period of up to three years of advanced study and research training in graduate departments and laboratories, either as candidates for the Ph.D. degree or as investigators obtaining postdoctoral research training in the biomedical sciences. Faculty Fellows are expected to return to the nominating institutions at the completion of such training.

MARC Faculty Fellows are selected on a competitive basis. Peer review of applications is provided by the MARC Review Committee, which is composed of consultants, including minority representatives from appropriate scientific fields, chosen primarily from the academic community. Evaluation is based upon the applicants' qualifications and potential for research and teaching as evidenced by academic records, reference reports and publications as well as by a research training proposal, the proposed training situation (i.e., the sponsor's and the institution's training facilities and staff), and other relevant information.

The second of the three support mechanisms, the MARC Visiting Scientist Award, has as its purpose the provision of financial support which allows outstanding scientist-teachers to serve in the capacity of visiting scientists at four-year colleges, universities, and health professional

schools where student enrollments are drawn substantially from minority groups. The primary intent is to strengthen research and teaching programs in the biomedical sciences for the benefit of students and faculty in these institutions by drawing upon the special talents of scientists from other institutions. Reciprocal benefits should accrue to the MARC awardee through the added experience gained by involvement in innovative science education and research development programs.

An applicant's proposal must include arrangements for the visiting scientist to reside in the campus community and to participate fully in programs of teaching, development of research, and/or counseling as outlined by the institution. Evidence of negotiations and of some tentative agreement between the applicant institution and the scientist-teacher nominated to serve as the MARC Visiting Scientist should be provided in the application. Individuals nominated as Visiting Scientists in this program should be recognized biomedical scientist scholars. Proposals may request support for the MARC Visiting Scientist Awards for periods ranging from an academic quarter to a maximum of one year. Stipends are determined on an individual basis according to the nominee's current salary or other possible source of stipend support for the proposed period in residence.

The third mechanism of support and the newest component of the Program is the MARC Honors Undergraduate Research Training Grant Program. This activity was initiated at the suggestion of the Congress, Institute consultants and staff, as a means of emphasizing the value and importance of providing biomedical training at the undergraduate level in minority institutions. The objective of the program is to increase the number of well-prepared students who can compete successfully for entry into graduate programs leading to the Ph.D. degree in the biomedical sciences, to help develop a strong science curriculum, and to stimulate an interest in undergraduate research as preparation for graduate study in the biomedical sciences.

HIGHLIGHTS

In April of 1977, the Institute was allowed to include prebaccalaureate training in predoctoral training awards granted under the aegis of the MARC Program. The Honors Undergraduate Program was announced in April 1977 with a request for applications to be submitted by mid-May 1977. A total of 67 applications were received for final review by the National Advisory General Medical Sciences Council in the fall of 1977. This activity resulted in 29 approvals, 33 disapprovals and 5 deferrals. A special meeting of the National Advisory General Medical Sciences Council was held in August 1977 to review these applications. Twelve awards were made in 1977 and 4 additional awards were made at the beginning of FY 1978. These awards totalled more than 1.7 million dollars. These first grants were awarded to institutions representing American Indians, Blacks, Hawaiians, and Mexican-Americans.

A second receipt date of February 1, 1978 was subsequently announced. The Program received 43 applications in response to this announcement. The staff was able to site visit 38 of the applicant institutions. Initial review was provided by the Minority Access to Research Careers Review Committee and the applications were scheduled for final review at the September, 1978 meeting of the National Advisory General Medical Sciences Council. To date, more than 140 such applications have been received for initial review and of these, 22 programs have been funded representing some 25 institutions. The awards will provide support for 157 trainees during FY 1980.

During FY 1979, the staff of the MARC Program, in its efforts to encourage all eligible minority institutions to participate in the activities of MARC, held workshops in various parts of the United States in order to communicate the general purposes and administrative requirements of the MARC Program, and specifically, those of the undergraduate component. Additionally, members of the Program Staff have held meetings in Bethesda with the Director and Executive Officers of the Federation of American Societies for Experimental Biology (FASEB), with the objective that exchanges of this sort would allow greater awareness of MARC activities. In this regard, a source of considerable satisfaction was that the MARC Program was represented by an exhibit, poster sessions, and a symposium during the 1979 FASEB meeting.

Because it was also considered advisable to address and to share problems and solutions which had arisen during the second year of undergraduate training grant support, a Program Directors' meeting was held in Dallas, Texas on May 3-4, 1979. Participants included both the Program Directors of funded Honors Undergraduate Research Training Grants and proposed directors of pending applications. The meeting agenda also included aspects of grants management and annual report procedures.

Program staff, in their continuing effort to improve the program, have undertaken a major evaluation of these components of MARC in order to assess improvements and accomplishments. This activity is being conducted in a cooperative manner, utilizing the minority scientific community itself as part of the evaluation process.

MARC Staff is currently compiling data which will provide the basis for decisions regarding the future directions of the MARC Honors Undergraduate Research Training Program. In particular, this should give some indication of whether the Honors Undergraduate Program is reaching its stated goals. This evaluative material is to be presented to the National Advisory General Medical Sciences Council in February 1980.

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